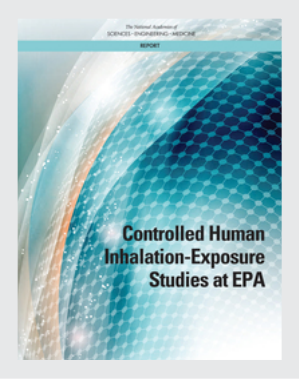

Controlled Human Inhalation-Exposure Studies at EPA

DETAILS

158 pages | 8.5 x 11 | PAPERBACK
ISBN 978-0-309-45249-6 | DOI 10.17226/24618

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Division on Earth and Life Studies

A Report of

The National Academies of

SCIENCES • ENGINEERING • MEDICINE

THE NATIONAL ACADEMIES PRESS

Washington, DC

www.nap.edu

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This activity was supported by Contract No. EP-C-14-005 from the U.S. Environmental Protection Agency. Any opinions, findings, conclusions, or recommendations expressed in this publication do not necessarily reflect the views of any organization or agency that provided support for the project.

International Standard Book Number-13: 978-0-309-45249-6
International Standard Book Number-10: 0-309-45249-X
Digital Object Identifier: 10.17226/24618

Additional copies of this publication are available for sale from the National Academies Press, 500 Fifth Street, NW, Keck 360, Washington, DC 20001; (800) 624-6242 or (202) 334-3313; http://www.nap.edu.

Printed in the United States of America

Suggested citation: National Academies of Sciences, Engineering, and Medicine. 2017. *Controlled Human Inhalation-Exposure Studies at EPA*. Washington, DC: The National Academies Press. doi: 10.17226/24618.

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Preface

The U.S. Environmental Protection Agency (EPA), as part of its efforts to obtain important scientific information for the periodic review of National Ambient Air Quality Standards (NAAQS) for criteria pollutants, conducts studies in which human volunteer participants are intentionally exposed by inhalation to pollutants under controlled experimental conditions. The objective of those studies has been to produce transient and reversible biomarker or physiologic responses that inform about biologic mechanisms of pollutant effects but do not cause clinical effects.

Citing evidence of causal relationships between air-pollutant exposures and human health effects or premature mortality, some members of Congress and others have expressed concern about the risk of those controlled exposures to the subjects who participate in such exposure studies. In 2012 the chairman of the House Subcommittee on Investigations and Oversight asked EPA's Office of Inspector General (OIG) to assess whether the agency followed applicable laws, regulations, policies, procedures, and guidance when it exposed human subjects to concentrated airborne particles or diesel exhaust particles.[1]

The OIG report concluded that EPA followed applicable regulations in conducting controlled human exposure studies of concentrated airborne particles or diesel exhaust particles. The report also made several recommendations to EPA for enhancing the protection of study subjects through the agency's policies and guidance.[2] In addition to implementing corrective actions in response to the OIG recommendations, EPA sought independent expert advice from the National Academies of Sciences, Engineering, and Medicine (NASEM) to address scientific issues and provide guidance on the conduct of controlled human exposure studies. In response, NASEM established the Committee on Assessing Toxicologic Risks to Human Subjects Used in Controlled Exposure Studies of Environmental Pollutants. The committee was asked to:

- Assess the utility of controlled inhalation exposure studies to inform and reduce uncertainties in setting air pollution standards to protect public health, and assess whether continuation of such studies is warranted.
- Assess health risks to test subjects who participated in recent studies of air pollutants at EPA's clinical research facility.
- If the committee supports continued conduct of human exposure studies, provide further guidance on methods for estimating levels of risk in controlled human exposure studies.
- Provide advice on a template for characterizing reasonably foreseeable risks, which could be used in obtaining informed consent from potential study participants. (The committee's formal statement of task is presented in Chapter 1.)

NASEM assembled a committee of 15 members who had expertise in clinical research trials, pulmonology, cardiology, critical care medicine, emergency medicine and medical toxicology, inhalation toxicology, ethics, exposure assessment, risk assessment, risk perception and communication, epidemiology, biostatistics, and environmental law. The committee included members knowledgeable about the different types of health-related evidence considered by EPA in the review of NAAQS. (Committee members' biographical information is presented in Appendix A.) I am grateful to the members of the committee for their efforts throughout this study.

[1]Letter, dated October 18, 2012, from Rep. Paul Broun, Chairman, Subcommittee on Investigations and Oversight, U.S. House of Representatives Committee on Science, Space, and Technology to Arthur A. Elkins, Jr., Inspector General, U.S. EPA.

[2]EPA. 2014. Improvements to EPA Policies and Guidance Could Enhance Protection of Human Study Subjects. Office of Inspector General. Report No. 14-P-0154. March 31.

In the course of preparing its report, the committee held a public information-gathering session on June 1, 2015, and heard presentations from EPA representatives. The committee also held an information-gathering session on August 24, 2016, and heard presentations from individuals outside of EPA (see Appendix B). The committee considered the information provided in both sessions in preparing its report.

In addition, the committee requested written information to describe various aspects of the controlled human inhalation exposure studies conducted at EPA's Human Studies Facility, located in Chapel Hill, North Carolina. Also, the committee requested information on how the results of those studies are used to inform agency decisions about the NAAQS. The committee also considered the OIG report, relevant previous NASEM reports, materials submitted by participants in the committee's August 2016 information-gathering session, applicable government regulations and policies, and other pertinent published and unpublished documents.

This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise. The purposes of the independent review are to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following for their review of this report:

George Annas, Boston University
Paul Appelbaum, Columbia University Medical Center/NY State Psychiatric Institute
Jessica Berg, Case Western University
Jiajing Chen, Saint Louis University
Jack Harkema, Michigan State University
Ilias Kavouras, University of Alabama
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James Merchant, The University of Iowa
Richard Smith, University of North Carolina
Sverre Vedal, University of Washington School of Public Health
Marsha Wills-Karp, Johns Hopkins Bloomberg School of Public Health

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of the report was overseen by the review coordinator, Joshua Sharfstein, Johns Hopkins Bloomberg School of Public Health, and the review monitor, Mark Cullen, Stanford University. They were responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the report rests entirely with the committee and the institution.

Finally, I wish to express my appreciation to the members of the project staff for the very effective support they provided to the committee.

Robert A. Hiatt, *Chair*
Committee on Assessing Toxicologic Risks to Human Subjects Used in Controlled Exposure Studies of Environmental Pollutants

Abbreviations

AHA	American Heart Association
AHRQ	Agency for Healthcare Research and Quality
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
CAA	Clean Air Act
CAP	Concentrated airborne particle
CASAC	Clean Air Scientific Advisory Committee
CHIE	Controlled human inhalation exposure
COPD	Chronic obstructive pulmonary disease
CV	Cardiovascular
DEP	Diesel engine exhaust particle
EC	Elemental carbon
EPA	US Environmental Protection Agency
ESC	Exposure scenario comparator
FA	Filtered air
GSTM1	Glutathione-*S*-transferase M1
HHS	Department of Health and Human Services
HRV	Heart rate variability
IRB	Institutional Review Board
ISA	Integrated Science Assessment
LAIV	Live attenuated influenza virus
MeS	Metabolic syndrome
MZ	Monozygote
NAAQS	National Ambient Air Quality Standards
NASEM	National Academies of Sciences, Engineering, and Medicine
NESHAP	National Emission Standards for Hazardous Air Pollutants
NHEERL	EPA National Health and Environmental Effects Research Laboratory
NBAC	National Bioethics Advisory Committee
NO_2	Nitrogen dioxide
O_3	Ozone
OC	Organic carbon
OHRP	Office for Human Research Protections
OIG	EPA Office of Inspector General
ORD	EPA Office of Research and Development
OTC	Over the counter
PI	Principal Investigator
PM	Particulate matter
PSS4	4-Point Perceived Stress Scale
SAB	EPA Science Advisory Board
SACHRP HHS	Secretary's Advisory Committee on Human Research Protections
SILS	Single Item Literacy Screener
tPA	Tissue type plasminogen activator
UFP	Ultrafine particle
WSP	Wood smoke particle

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Summary

The Clean Air Act calls for the U.S. Environmental Protection Agency (EPA) administrator to "conduct studies, including epidemiological, clinical and laboratory and field studies as necessary to identify and evaluate exposure to, and effects of, air pollutants on human health." In carrying out that mandate, EPA's Office of Research and Development recruits human volunteers to participate in studies in which they are intentionally exposed to pollutants by inhalation under controlled experimental conditions.[1] EPA conducts controlled human-inhalation exposure (CHIE) studies, also referred to as human clinical studies or human challenge studies, at its Human Studies Facility on the campus of the University of North Carolina at Chapel Hill. The information is used primarily to inform the periodic review of the National Ambient Air Quality Standards (NAAQS) for criteria pollutants.

The objective of EPA CHIE studies has been to produce transient and reversible biomarker or physiologic responses (such as a temporary change in lung function) that inform about biologic mechanisms of pollutant effects but do not cause clinical effects. EPA uses the results to help understand pathways of toxicity by which air-pollutant exposures might lead to illness or premature death in sensitive groups in the U.S. population, such as groups of people who have heart or lung disease. The agency also uses the results to inform its air-quality management decision making.

In response to a congressional request in 2012, EPA's Office of Inspector General (OIG) assessed whether the agency followed applicable requirements when it exposed human subjects to concentrated airborne particulate matter (PM) or diesel exhaust particles. The request pointed to concerns about the intentional human exposure to those pollutants, given evidence of causal relationships between ambient exposures and human health effects or premature mortality. The OIG assessment concluded that EPA followed applicable regulations in conducting the studies. The OIG also made several recommendations to EPA, for example, that it define "reasonably foreseeable risks" and provide examples of them to study participants and that it develop procedures for ensuring that participant consent forms present exposure information and short- and long-term risks, including cancer risks, in a consistent manner.

THE COMMITTEE'S STUDY

In addition to implementing corrective actions in response to the OIG's recommendations, EPA asked the National Academies of Sciences, Engineering, and Medicine to establish a committee to assess the value of CHIE studies for informing and reducing uncertainties in setting air-pollution standards to protect public health. The committee also was asked to assess health risks to study subjects who had participated in recent EPA CHIE studies. And the committee was asked, if it supported the continued conduct of such studies, to provide further guidance on methods for estimating risks posed to study subjects and on characterizing reasonably foreseeable risks when seeking consent from people to participate in CHIE studies.[2]

[1]In this context, *controlled* refers to the aspect of experimental design intended to minimize the effects of factors other than the exposure conditions (that is, the type of pollutant, concentration, and duration) on the physiologic response measurements identified in the research protocol. The researcher varies the exposure conditions in a systematic way to assess the effect of changes in those conditions on the responses of interest.

[2]The committee's full statement of task is presented in Chapter 1.

Controlled Human Inhalation-Exposure Studies at EPA

THE DEVELOPMENT OF CONTROLLED HUMAN INHALATION-EXPOSURE STUDIES

In carrying out its task, the committee considered general requirements for ethical research and the previous 2004 National Research Council report *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues*, which presented conditions under which it is ethically acceptable to involve humans in studies that expose them to chemical toxicants that would result in somewhat higher risks than studies that pose no identifiable risks or for which there is a reasonable certainty of no harm.

CHIE study designs are developed by EPA scientists and reviewed by other agency staff and two outside experts to evaluate aspects of the safety of study subjects, scientific rigor, adherence to ethical principles, and the extent to which expected study results would support air quality–related decisions. All CHIE studies are required to receive prior approval from the University of North Carolina Institutional Review Board (IRB). IRB approval means that the net risks posed to the subjects have been determined to be justified by the study's expected social value. The IRB of record has the additional responsibility of monitoring the progress of the study and, if necessary, withdrawing its approval.

Study subjects are enrolled after it is determined, through EPA's preexposure health evaluations, that there is no reason to believe that their participation in the study will lead to an adverse event.[3] EPA applies a broad set of criteria when selecting study subjects. The health status of subjects is monitored shortly before, during, and immediately after the exposure studies and usually again about 24 hours later. If during a study there is any evidence that a person is being or has been harmed, the person is referred for medical observation or treatment. EPA investigators also are responsible for addressing adverse effects that become apparent only after a study is completed (see Chapter 5).

The committee concludes that EPA's procedures are consistent with and indicative of ethical approaches to human-subjects research; this conclusion is consistent with conclusions of the EPA OIG's report. The committee also concludes that CHIE studies have by design been limited to exposures of subjects that are highly unlikely to exhibit responses of adverse clinical significance by screening of potential subjects and selecting pollutants and concentrations that are not expected to produce adverse short-term responses. The latter selection is usually based on reports of associations in observational epidemiologic studies in larger populations or in studies of laboratory animals at comparable concentrations.

VALUE OF CONTROLLED HUMAN INHALATION-EXPOSURE STUDIES

The committee assessed the value of past CHIE studies by considering their scientific contributions and the societal benefit of providing a basis for NAAQS decisions. The Clean Air Act requires that EPA periodically review each of the NAAQS by evaluating the most recent evidence and uncertainties and then deciding whether the existing NAAQS are adequate to protect public health with an adequate margin of safety and whether they should be retained or revised in the light of new information. The committee focused primarily on the contributions of CHIE studies conducted by EPA and other organizations to the body of knowledge for the NAAQS of PM and the NAAQS for ozone (O_3) and related photochemical oxidants.

The committee concludes that CHIE studies have provided unique information that cannot be obtained from animal inhalation studies or from studies of people engaged in their normal daily activities (that is, through epidemiologic studies).

[3]An adverse event is any untoward or unfavorable medical occurrence in a human subject—including any abnormal sign (such as an abnormal physical examination or laboratory finding), symptom, or disease—that is temporally associated with the subject's participation in the research, whether or not it is considered to be related to the subject's participation in the research. A serious adverse event is one that is fatal or life threatening, results in substantial or persistent disability, requires or prolongs hospitalization, results in a congenital anomaly or birth defect, or indicates other important hazards or potentially serious harm to research subjects or others. (See Chapter 2 for formal definitions from the U.S. Department of Health and Human Services.)

Summary

The design of CHIE studies enables formal tests of hypotheses and less ambiguous assessments of short-term exposure–response relationships for specific laboratory-generated pollutants or mixtures. PM and O_3 CHIE studies have enabled investigators to separate the effects of exposure to individual criteria pollutants or specific groups of criteria pollutants from effects associated with exposures to ambient complex mixtures that are observed in epidemiologic studies. They have also enabled specific assessments of the timing of responses to short-term exposures to criteria pollutants.

Even though the generalizability of CHIE study results is limited by the studies' narrow hypotheses and use of small numbers of study subjects, the studies have played a key role in evaluating and elucidating biologic or physiologic mechanisms through which air pollutants might lead to health effects (without the intent or need to observe clinical health effects in the CHIE studies). CHIE studies have provided assessment of multiple biomarker and physiologic responses to specific criteria-pollutant exposures and thus enabled evaluation of potential mechanisms of short-term actions. Developing and refining biomarkers of responses to short-term inhalation exposures to specific pollutants can lead to incorporation of the biomarkers in epidemiologic studies and animal inhalation studies. CHIE studies have also helped to determine the utility of the biomarkers in studies of disease progression.

CHIE studies involving short-term exposures to O_3 have contributed to clarification of exposure–response relationships. However, CHIE studies of PM have not focused on clarification of exposure–response relationships; they have involved the use of fairly uniform exposure concentrations of PM that have particles of different sizes (coarse, fine, or ultrafine). The difference between the O_3 and PM study designs is due in part to the complication of PM studies by the geographically and temporally variable composition of ambient PM, which makes it more difficult to characterize exposure–response relationships unambiguously at the national level. Such a complication is not an issue with CHIE studies of single gaseous pollutants, such as O_3.

Combination of CHIE study results with information from epidemiologic and toxicologic studies can facilitate a holistic evaluation of the evidence and thereby provide a well-considered scientific basis for establishing or revising a NAAQS. Although the EPA CHIE study design generally precludes inclusion of subjects who have serious health conditions, the studies have helped to define an adequate margin of safety, as required in the Clean Air Act, and have begun to define the groups that have substantial risk factors associated with air-pollution exposures, including people who have heart or pulmonary disease and people of low socioeconomic status.

CHIE studies of O_3 have been of critical importance for the NAAQS by providing a number of advances, including

- A basis for EPA's decision to move from a 1-hour to an 8-hour averaging time for the O_3 NAAQS level (concentration), and
- Demonstrations of the importance of considering susceptibility factors and variability among individuals in human physiologic responses (such as changes in lung function) and biologic responses (such as increases in biomarkers of pulmonary inflammation) to exposure to O_3 and other oxidant pollutants.

CHIE studies of PM have been valuable in providing a number of advances, including

- Evidence on physiologic and biologic effects of exposures to high-PM mass-based concentrations, suggesting biologic plausibility of epidemiologic study results that demonstrate associations of ambient fine-PM mass-based exposures with clinical cardiovascular outcomes; and
- Confirmation in humans of PM-related biologic or physiologic effects observed in animal toxicity studies. Human studies and animal toxicity inhalation studies have been complementary in improving understanding of sources of sensitivity to PM mass-based exposures, and in exploring organ systems that are physiologically perturbed by PM exposure. As mentioned above, those studies have been complicated by the geographically and temporally variable composition of ambient PM.

Controlled Human Inhalation-Exposure Studies at EPA

POTENTIAL SOCIETAL BENEFITS OF FUTURE STUDIES

Well-designed CHIE studies could address needs in information relevant to the review of air-quality standards and the regulation of pollutant sources, such as information needed to fulfill the Clean Air Act mandate to protect public health, including the health of sensitive groups, with an adequate margin of safety. CHIE studies that address those gaps would have societal benefits by, for example, providing a greater understanding of the effect of PM composition on potential health responses and of the mechanisms by which people might exhibit sensitivity to air-pollution exposure.

RISKS TO PARTICIPANTS IN PAST CONTROLLED HUMAN INHALATION-EXPOSURE STUDIES

In assessing health risks to participants in recent EPA CHIE studies, the committee reviewed documents on the eight studies provided by EPA to illustrate current practices for articulating study objectives, rationale, and design with respect to the approaches and the substances being tested (see Box S-1). In addition, the committee assessed the adverse events reported to the University of North Carolina IRB for all CHIE studies for the period from January 2009 to February 2015.

Risks of adverse events temporally associated with a subject's participation in a CHIE study might be affected by one or more of the following:

- Air-pollutant exposures occurring independently of the study several days before or during the multiday experiment,
- Intended pollutant exposures during the experiments,
- Subjects' preexisting medical conditions or sensitivities to study pollutants,
- Other experimental procedures during the study (such as blood sampling or bronchoscopy), and
- Pathophysiologic events (such as a serious adverse cardiac or pulmonary event) that, although unrelated to air-pollutant exposures, might happen to occur in subjects during the study.

The biologic responses of CHIE study subjects that were anticipated by the study protocols dissipated after cessation of the exposures and were not known to have constituted any long-term adverse consequence for the health of any of the participants. For the period January 2009 to October 2016, the CHIE studies conducted at EPA's Human Studies Facility involved 845 intentional exposures to pollutants and 555 exposures to clean air. Of those exposures, only one resulted in the hospitalization of a study subject. The subject experienced an unexpected serious event of paroxysmal atrial fibrillation a very short time after being exposed to concentrated ambient PM during the XCON study. The subject's response reverted to normal sinus rhythm spontaneously without clinical sequelae about 2 hours after cessation of the pollutant exposure. The subject was observed overnight in the hospital after the event. The occurrence of one hospitalization, which corresponds to 0.1% of the pollutant exposures, illustrates that, despite substantial efforts to screen potential study subjects, some level of risk is present.

In light of its review of eight CHIE studies, the committee finds that the risk of a serious adverse event with long-term sequelae is unlikely to be large enough to warrant concern but recognizes that it is never possible to conclude that there is no risk.

Specific concerns have been expressed about CHIE-study risks of chronic diseases, such as lung cancer and ischemic heart disease, which correlate with long-term cumulative exposure to $PM_{2.5}$ (particles with an aerodynamic diameter less than or equal to 2.5 μm). However, because those diseases are considered to be associated with cumulative effects that develop over long periods, $PM_{2.5}$ exposures in the CHIE studies considered by the committee (for example, up to 600 $\mu g/m^3$ for 2 hours) would add very little to the cumulative lifetime $PM_{2.5}$ exposures of many people in the United States. That suggests that any increase in chronic disease risk resulting from $PM_{2.5}$ CHIE exposures in the studies considered by the committee would be vanishingly small.

BOX S-1 Eight Studies Identified by EPA for Consideration by the Committee

Cardiopulmonary Responses to Exposure to Ozone and Diesel-Engine Exhaust with Moderate Exercise in Healthy Adults (DEPOZ)

To examine whether coexposures to O_3 and diesel-engine exhaust (DE), at concentrations in the upper range of those encountered in urban settings, can induce additive or synergistic biologic responses and whether previous DE exposure can alter response to O_3 exposure.

Effects of Sequential Exposure to Nitrogen Dioxide and Ozone in Healthy Adult Human Volunteers (ENDZONE)

To determine whether exposure to O_3 or nitrogen dioxide (NO_2) increases cardiopulmonary responses of healthy adults to a subsequent exposure to the other pollutant relative to exposure to either pollutant without a subsequent exposure.

Epigenetic Effect Modifications with Ozone Exposure on Healthy Volunteers (GEMINOZ)

To determine whether differences in baseline epigenetic profiles between subjects are associated with responsiveness to O_3 exposure and whether O_3 exposure itself causes acute changes in a subject's epigenome. *Epigenetic* refers to mechanisms not involving changes in DNA sequence that influence gene expression.

Mechanisms by which Air Pollution Particles Exacerbate Asthma in Older Adults with Mild Asthma (KINGCON)

To study effects of inhaled pollutants in relation to the glutathione-*S*-transferase M1 (GSTM1) genotype in older adults who have asthma. Previous studies have indicated that asthmatics who have the null genotype for GSTM1 have increased susceptibility to O_3 and DE exposures.

Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles (OMEGACON)

To determine whether fish oil containing omega-3 fatty acids reduces the respiratory and cardiovascular effects of PM.

The Interaction of Social Factors with Air Pollution (SOZIAL)

To study effects of psychosocial stress on health responses to O_3 exposures and help to understand which groups and individuals are at increased risk from air pollution.

Effects of Wood Smoke Particles on Influenza-Induced Nasal Inflammation in Normal Volunteers (WOODSIE)

To study the pathophysiology of the association between exposure to PM and the likelihood of a viral infection and the response to that infection.

Physiologic Changes in Adults with Metabolic Syndrome Exposed to Concentrated Ultrafine Chapel Hill Air Particles (XCON)

To examine biologic responses to exposure to concentrated ambient ultrafine particles exposure in patients who have metabolic syndrome, a collection of risk factors (such as high blood pressure) that increase the likelihood of developing cardiovascular disease or type-2 diabetes mellitus.

Controlled Human Inhalation-Exposure Studies at EPA

THE CONTINUED CONDUCT OF CONTROLLED HUMAN INHALATION-EXPOSURE STUDIES BY THE ENVIRONMENTAL PROTECTION AGENCY

Having evaluated the historical contributions of CHIE studies, human-subjects study protocols, the likelihood of serious adverse events with long-term sequelae, and the potential for societal benefits, the committee concludes that the continued conduct of EPA CHIE studies is warranted, with the improvements discussed in this report.

EPA CHIE studies should continue to be undertaken cautiously under two conditions: (1) only when a CHIE study is expected to provide additional knowledge that informs policy decisions and regulation of pollutants that cannot be obtained by other means and (2) when it is reasonably foreseeable that the risks for study participants will not exceed transient and reversible biomarker or physiologic responses.

Selection of Study Subjects

In using CHIE studies to assess biologic plausibility of a pollutant-related effect or sensitivity to pollutant exposures, involvement of study subjects from groups with stable chronic conditions hypothesized to exhibit increased biomarker responses or physiologic effects of pollution (but not adverse events) might be more informative than involvement of healthy young adults. Therefore, the committee strongly supports EPA's use of the process of medical screening of potential volunteers, thus ensuring that the health assessment is not limited to a volunteer's knowledge of his or her own health status. In addition, the committee offers the following recommendations to ensure the continuation of various important activities and initiate several new ones regarding future CHIE studies. (See Chapter 5 for additional recommendations.)

EPA should continually review and update its risk-profile information on groups that exhibit sensitivity to air-pollutant exposures to inform decisions on inclusion and exclusion criteria for the selection of CHIE study subjects.

EPA and IRBs should determine which sensitive groups are appropriate for CHIE studies, keeping in mind that appropriate expected outcomes of CHIE studies are biomarker or physiologic outcomes but not adverse outcomes.

EPA should exclude potential study participants if they are in a sensitive group known to be at increased risk of a serious adverse event (such as people who have had myocardial infarction). Investigators should use up-to-date approaches (such as the use of validated and calibrated risk-stratification tools developed by the American Heart Association and the Reynolds risk score) for grouping or stratifying potential participants according to their background risk of adverse events.

EPA investigators should continue to review the most recent animal and human toxicologic literature and human epidemiologic literature to evaluate safety when considering future CHIE studies, with particular attention to studies that would involve exposures to pollutant mixtures that had been included in few or no previous CHIE studies.

For CHIE studies involving exposures to novel pollutant combinations, EPA should evaluate the safety of the exposure concentrations by conducting dose-escalation studies that initially involve low exposure concentrations and a small number of subjects and EPA should provide sufficient time for follow-up before involving a larger number of subjects.

In addition to its IRB reporting, EPA should document all serious adverse events associated with participation in CHIE studies and the actions taken in response to them.

Additional External Scientific Input

Documents on the eight studies allowed the committee to gain some perspective on scientific priorities of EPA investigators for future CHIE studies and their rationale for the priorities. Although the committee concludes that EPA CHIE studies are addressing important questions, it has concerns about the adequacy of the process for ensuring the most important CHIE study topics are selected and the external scientific input to maximize the rigor and value of each CHIE study. EPA would benefit by augmenting the process by which CHIE study topics are selected to obtain a broader array of input from the external scientific community on the importance and merit of the question being addressed and considerations that would ensure scientific validity. The external input could also help to strengthen coordination of EPA's epidemiologic and toxicologic resources with those of its Human Studies Laboratory to ensure that scientific progress in each field informs future research plans and that understanding of participant risk factors and the potential value of the CHIE studies continue to be improved. The committee envisions that this external scientific input would be nonbinding on EPA or the reviewing IRBs, but the input would be provided in advance of IRB review and internal EPA review.

EPA should convene an external scientific advisory committee of experts on a regular basis to review the agency's progress and provide advice on the creation of a portfolio of CHIE studies with the objectives of breaking new scientific ground relevant to Clean Air Act mandates and ensuring protection of human subjects.

CHARACTERIZING RISKS TO SUBJECTS IN FUTURE STUDIES

Health risks to participants cannot be assumed to be the same for each CHIE study. Risks will vary according to study design (such as exposure agent, concentration, and duration) and the health status or risk profile of the individual participants. Once potential adverse outcomes are identified according to the principle of reasonably foreseeable risks and some decision is made about whether these outcomes are likely to be acute or chronic, risk characterization is necessary. There are two possible methods for characterizing risks of acute and chronic effects:

- Quantitative approaches that obtain a risk estimate from a published epidemiologic study of ambient-air exposures and adjusts the estimate according to the exposure duration of the CHIE study relative to the duration in the epidemiologic study, and
- The use of an exposure scenario comparator (ESC) approach, which involves comparing experimental exposure concentrations and durations with readily recognizable scenarios of real-world exposures at ambient concentrations of similar magnitude and duration experienced by a population in everyday life at a particular location.

The committee recommends that risk-characterization objectives be addressed by using an ESC approach in which the risk associated with a CHIE-study exposure is likely to be lower than the risk to the comparative population.

Although EPA researchers and IRBs of record might seek quantitative estimates of risk of potential acute effects (for example, to estimate the increment that a CHIE study might add to the baseline risk associated with exposure to ambient air pollution), the committee considers the ESC approach to be the better alternative overall. Given the limited availability of appropriate data for risk calculations, the large attendant uncertainty in the results, (particularly that stemming from attempting to estimate risks from

very short-term low exposures from data in which subjects are exposed to higher levels for much longer periods of time) and uninitiated people's potential difficulty in understanding the implications of the quantitative results in the context of controlled short-term exposures, the committee focused on the ESC approach. Such an approach would be useful to investigators, IRBs, and potential participants in characterizing acute and chronic risks.

An ESC approach would provide a useful context for considering chronic effects of the air pollutants being studied, which are thought to be associated with cumulated results of long-term exposures developed over long periods. In considering chronic effects, 2–4 hours of participation in a CHIE study would add only incrementally to the cumulative ambient background exposure over the life of a person, and calculating a risk estimate would involve so much uncertainty that the estimate would have little meaning. Therefore, the committee strongly prefers the use of an ESC approach for the characterization of risks related to participation in a CHIE study.

To illustrate that the risk associated with participation in a CHIE study is likely lower than the risk to the comparative population, the comparative scenario should involve a documented ambient exposure concentration that is higher than the exposure concentration in the CHIE study and an exposure duration in the comparative scenario that is at least as long as the experimental exposure duration.

It is not advisable to compare a lower ambient concentration over a longer period with the CHIE study's concentration and duration. Attempting to represent an equivalent ambient exposure in that way introduces more uncertainty as to whether the risk in the comparative scenario is greater than that in the CHIE study.

The comparative exposure scenarios should be documented fully so that the reasonableness of the comparison can be evaluated. Comparative exposure scenarios should be based on populations in the United States, insofar as possible. When that is not feasible, scenarios involving populations with demographics and life styles as similar as possible to those in the United States should be given precedence.

EPA has compared the exposures to the various pollutants used in the eight CHIE studies with exposures to the pollutants that might be experienced by various groups of people living in the United States. However, many of the comparison exposure scenario comparators in the CHIE studies were undocumented, and ones that were documented were not all appropriate, for example, because of the substantial differences in exposure durations between the scenario and the CHIE study protocol.

In planning a CHIE study, EPA should obtain the appropriate monitoring data for exposure scenario comparators by using locations where populations are or were exposed to ambient concentrations exceeding the exposure concentration envisioned for the study.

Insofar as possible, the comparative scenario ought to be one that the participants in the CHIE study can readily identify with and understand. However, recent improvements in U.S. air quality might make it difficult to find recent ambient concentrations that are appropriate for use in an exposure scenario. Instead, the scenario could include a population at a U.S. location in the past or a present population in another country. If a particular exposure regimen has been used numerous times in previous CHIE studies without the occurrence of adverse effects, the accrued experience from those studies could be useful in developing a reasonable exposure comparator.

It is important to note that if a CHIE study is being proposed for which no appropriate ambient concentrations (past or present) can be found and no previous CHIE studies that found no adverse effects are applicable, it might be an indication that the CHIE study requires further explicit justification or should not be conducted.

COMMUNICATION WITH POTENTIAL STUDY SUBJECTS ABOUT INFORMED CONSENT

For a person considering participation in a CHIE study, information about the study is presented through a disclosure process. The potential participant considers the information in a deliberative process and decides to participate or not to participate. Some of EPA's informed-consent documents reviewed by the committee contain complicated and technical language that requires high literacy and numeracy skills. In addition, comparative exposure scenarios presented in those documents might not be familiar or relevant to participants.

EPA should use a plain-language presentation of risk information in consent documents for all IRB protocols. The agency should characterize reasonably foreseeable risks by using an easily understood perspective and incorporating relevant exposure comparator scenarios into language about the study. The comparators should be evidence based and their development explained

Characterization of reasonably foreseeable risks is an especially important part of disclosure to potential participants. The committee agrees with the approach taken by EPA in designating a risk as reasonably foreseeable if there is some credible evidence that harm might occur. However, an overdetailed list of all possibilities can result in a less valid consent process in that it groups the anticipated or likely risks with ones that are only distant possibilities.

In its report, the committee provides recommendations on consent-document development and on assessing participant comprehension of the documents (see Chapter 7). It provides the following recommendations for the development of consent documents with respect to reasonably foreseeable risks and other risks of possible concern. EPA should

- **Provide accumulated information on the occurrence of serious adverse events associated with previous CHIE studies and on the resolution of the events to illustrate that a study involves risks of serious adverse events that can be anticipated and those that cannot be anticipated;**
- **Describe uniformly the risks from experimental procedures that are used often (such as bronchoscopy) and indicate how the risk profile of study subjects (such as mild asthmatic) has been taken into account; and**
- **Include and delineate all reasonably foreseeable risks and any risks likely to be perceived as important by the participants.** CHIE studies typically impart a very small increase in the cumulative exposure to ambient air pollution over a person's lifetime, and there is no credible evidence to suggest that chronic effects should be considered among the reasonably foreseeable risks in the studies. However, because of associations between long-term exposure to air pollution and chronic effects, the likelihood of chronic effects needs to be included in informed-consent communications, such as by using the recommended ESC approach for characterizing risks (see above). Allowing people to judge risks for themselves and determine if they are willing to assume those risks is essential in respecting the autonomy of participants.

CONCLUDING REMARKS

The committee concludes that CHIE studies have provided unique information that helps to enrich scientific understanding of underlying physiologic short-term responses to daily inhalation exposures to airborne pollutants or mixtures thereof. Such information is important for future NAAQS reviews. The committee judges that in the eight CHIE studies that it reviewed any risks of a serious adverse event with long-term sequelae were unlikely to be large enough to be of concern, although it should never be concluded that no risk was possible. The committee concludes that the continued conduct of EPA CHIE studies is warranted, with improvements in human-subjects oversight, protocols, consent forms, and commu-

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nication with potential participants during the informed-consent process and improvements in scientific oversight to maximize the potential for the societal benefits of the studies. EPA CHIE studies should continue to be undertaken cautiously under two conditions: (1) only when a CHIE study is expected to provide additional knowledge that informs policy decisions and regulation of pollutants that cannot be obtained by other means and (2) when it is reasonably foreseeable that the risks for study participants will not exceed transient and reversible biomarker or physiologic responses.

1

Introduction

The U.S. Environmental Protection Agency (EPA) has a mission and regulatory responsibility to protect human health and the environment. The Clean Air Act (CAA) is one of the major federal pollution-control laws through which EPA carries out its mission, and has as one of its main goals "To initiate and accelerate a national research and development program to achieve the prevention and control of air pollution" [USC 7401(b)]. The CAA also indicates that the EPA administrator "shall conduct studies, including epidemiological, clinical and laboratory and field studies as necessary to identify and evaluate exposure to, and effects of, air pollutants on human health" [USC 7403(d)].

EPA's pursuit of that goal includes a variety of research activities involving human subjects, such as epidemiologic studies and surveys. Those research activities also involve studies of individuals who volunteer to be exposed to air pollutants intentionally in controlled laboratory settings so that measurements can be made of transient and reversible biomarker or physiologic responses to those exposures that can indicate pathways of toxicity and mechanisms of air-pollution responses. The results of those controlled human inhalation exposure (CHIE) studies, also referred to as human clinical studies or human challenge studies, are used to inform policy decisions and help establish or revise standards to protect public health and improve air quality.

In the CHIE study context, *controlled* refers to the aspect of experimental design intended to focus on exposure conditions (that is, the type of pollutant, concentration, and duration) and minimize the effects of other factors on the physiologic response measurements identified in the research protocol. The researcher varies the exposure conditions in a systematic way to assess the effect of changes in those conditions on the responses of interest.

A PRIOR NATIONAL RESEARCH COUNCIL REPORT ON CONTROLLED HUMAN EXPOSURE STUDIES

In 2004, the National Research Council (NRC) issued a report on *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues* (NRC, 2004a). The report was prepared in response to a request from EPA to review the ethical and scientific issues posed by the agency's possible use of third-party studies (conducted by organizations outside of EPA) that intentionally exposed humans to toxicants to identify or quantify their effects. The impetus for that study stemmed from the use of human studies to inform regulatory decisions concerning agricultural pesticides. There was concern that the companies conducting the health effects research had a financial stake in the outcome of the toxicity testing, resulting in a conflict of interest. Another concern was whether it is ethical to conduct research that involves the exposure of healthy volunteers to chemicals for the purpose of assisting EPA in setting regulatory standards for the general public.

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The 2004 NRC report recommended that intentional exposure studies in humans be conducted and used for EPA regulatory purposes only if all the following conditions are met:

- The study is necessary and scientifically valid.
- The societal benefits of the study outweigh any anticipated risks to participants.
- There is reasonable certainty that participants will experience no adverse effects.
- All of the recognized ethical standards and procedures for protecting the interests of study participants are observed.

The committee is in agreement with that report. Selected recommendations that are directly relevant to this present study are provided in Box 1-1.

Subsequent to the 2004 report, EPA established the Human Studies Review Board to review third-party controlled human-exposure studies submitted by the Office of Pesticide Programs in the Office of Chemical Safety and Pollution Prevention (EPA, 2016a). The board was not chartered to review CHIE studies carried out by EPA. However, the agency has other processes in place to review CHIE studies, as discussed below and in Chapter 2.

CHIE STUDIES AT EPA

Research involving human subjects to study exposure to and effects of air pollutants on health is conducted by the National Health and Environmental Effects Research Laboratory (NHEERL) of EPA's Office of Research and Development. EPA's CHIE studies are carried out at EPA's Human Studies Facility in Chapel Hill, North Carolina. EPA conducts CHIE studies involving subjects who are healthy individuals or those with mild medical conditions who are considered unlikely to have a serious adverse health response to the controlled exposures. The regulations that govern human research at EPA provide the general requirements for informed consent the agency must follow in seeking the involvement of human subjects in a CHIE study (40 CFR 26A). EPA CHIE studies cannot be conducted on children or pregnant or nursing women (40 CFR 26B). The potential effects to be observed in the individuals who participate in these studies are expected to be transient and reversible. Researchers use the results to help understand pathways of toxicity by which air-pollutant exposures might lead to illness or premature death in at-risk (or sensitive) groups in the U.S. population, such as individuals with heart or lung disease (EPA, 2016b).

According to EPA, the agency initiates CHIE studies when there is evidence that only reversible biologic changes will occur during and following a planned exposure based on prior data from one or more of the following types of research:

- Testing in laboratory animals,
- Observational research involving only naturally occurring human exposures (that is, exposures occurring as humans go about their normal activities in their regular environments), or
- CHIE studies of a very closely related substance or mixture.

As discussed in Chapter 2, EPA's CHIE studies must comply with the "Common Rule," a set of regulations that govern the ethical and scientific conduct of federally supported research with human subjects. Before a CHIE study can begin, the research must undergo multiple layers of review and approval, involving several officials within the agency, two outside scientists (usually research physicians), and an external Institutional Review Board (IRB). See EPA (2017a) for additional information on safeguards for human-subjects research.

BOX 1-1 Selected Recommendations from *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues* (NRC, 2004a)

Establishing Scientific Acceptability

The scientific and ethical considerations of human participants' research are closely related. Research that deliberately exposes humans to toxicants must be both scientifically and ethically justified. Such a study could be scientifically valid but ethically unacceptable (e.g., because the investigator failed to get informed consent or exposed participants to too much risk); however, a study cannot be ethically acceptable if it is scientifically invalid. A sound research design is the first step in developing an ethically acceptable protocol. For these reasons, scientific and ethical considerations should be integrated in the review and evaluation of all human research studies.

Scientific Validity of Intentional Human Dosing Studies

EPA should issue guidelines for determining whether intentional human dosing studies have been

a. Justified, in advance of being conducted, as needed and as scientifically appropriate, in that they could contribute to addressing an important scientific or policy question that cannot be resolved on the basis of animal data or human observational data;
b. Designed in accordance with current scientific standards and practices to (i) address the research question, (ii) include representative study populations for the endpoint in question, and (iii) meet
c. requirements for adequate statistical power;
d. Conducted in accordance with recognized good clinical practices, including appropriate monitoring for safety; and
e. Reported comprehensively to EPA, including the full study protocol, all data produced in the study (including adverse events), and detailed analyses of the data.

Balancing Risks and Benefits Value of Studies That Seek to Provide a Potential Public Health or Environmental Benefit

An IRB should be properly constituted to be able to consider whether a study has the potential of providing a clear health or environmental benefit to the community. Such studies could be acceptable even if they involved a somewhat higher level of risk than that posed by studies for which there is no identifiable risk or for which there is a reasonable certainty of no harm. No study is ethically justifiable if it is expected to cause lasting harm to study participants.

Criteria for Scientific and Ethical Acceptability

Studies that do not meet the highest scientific and ethical standards should not be carried out or accepted by EPA as input to the regulatory decision-making process. Necessary conditions for scientifically and ethically acceptable intentional human dosing studies include

a. Prior animal studies and, if available, human observational studies;
b. A demonstrated need for the knowledge to be obtained from intentional human dosing studies;
c. Justification and documentation of a research design and statistical analysis that are adequate to address an important scientific or policy question, including adequate power to detect appropriate effects;
d. An acceptable balance of risks and benefits and minimization of risks to participants;
e. Equitable selection of participants (for example, the selection of research participants should avoid exploitation of any particular social group);
f. Free and informed consent of participants; and
g. Review by an appropriately constituted IRB or its foreign equivalent.

Participant Selection Criteria

IRBs reviewing intentional human dosing studies should ensure that the following conditions are met in selecting research participants:

a. Selection should be equitable.

(Continued)

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BOX 1-1 Continued

b. Selection of persons from vulnerable populations must be convincingly justified in the protocol, which also must justify the measures to be taken to protect those participants.
c. Selection of individuals with conditions that put them at increased risk for adverse effects in such studies must be convincingly justified in the protocol, which also must justify the measures that investigators will use to decrease the risks to those participants to an acceptable level.

Best Practices in Informed Consent

EPA should develop and disseminate to relevant IRBs, investigators, and sponsors a list of best practices regarding informed consent in intentional human dosing studies. EPA should encourage all sponsors and investigators to adopt these practices, and it should require their adoption in studies it sponsors or conducts.

Compensation for Research-Related Injuries

At a minimum, sponsors of or institutions conducting intentional human dosing studies should ensure that participants receive needed medical care for injuries incurred in the study, without cost to the participants.

In addition, EPA should study whether broader compensation for research-related injuries should be required.

Creation of a Comprehensive EPA Human Studies Review Process

EPA should require that all human research conducted for regulatory purposes be approved in advance by an appropriately constituted IRB or an acceptable foreign equivalent. Research conducted by EPA scientists should be reviewed by an EPA-authorized IRB.

Human Studies Review Board

To ensure that intentional human dosing studies conducted for EPA regulatory purposes meet the highest scientific and ethical standards, EPA should establish a Human Studies Review Board to address in an integrated way the scientific and ethical issues raised by such studies. To the extent possible, this board should review in a timely manner the protocols and the justification for all intentional dosing studies intended for submission to EPA, as well as study results when completed. These reviews should be conducted regardless of the sponsor or site of performance, and EPA should communicate the results of the reviews to relevant parties.

Review of the Human Studies Review Board

The proposed Human Studies Review Board, its functions, and its record should be assessed after 5 years by a body composed of EPA staff and external reviewers.

EPA'S OFFICE OF INSPECTOR GENERAL REPORT

In 2012, a congressional request for an Office of Inspector General (OIG) review was focused specifically on CHIE studies performed at the EPA Human Studies Facility in Chapel Hill, North Carolina (Broun, 2012). The review request asked whether the agency followed applicable laws, regulations, policies, procedures, and guidance when it exposed human subjects to concentrated airborne particulate matter (PM) or diesel exhaust particles. OIG reviewed the conduct of five studies that were carried out in 2010 and 2011 and found that the facility had complied with all applicable regulations, guidance, and policies (EPA, 2014a). OIG also identified some areas where clearer policies and guidance would enhance protection of study subjects, and suggested modifications to procedures involving obtaining approvals,

informed consent procedures, and monitoring adverse events. OIG's report included these recommendations:[1]

- Obtaining approvals. OIG recommended that NHEERL revise its human research guidance to indicate the review and approval process for significant study modifications, implement procedures to document that study investigators have met the requirement for annual ethics training, and develop management controls to ensure that reviews and approvals are documented and following NHEERL guidance.
- Informed consent. OIG recommended that NHEERL define and provide examples of "reasonably foreseeable risks" and develop procedures to ensure that consent forms present exposure information and short- and long-term risks, including cancer risks, in a consistent manner.
- Adverse events. NHEERL guidance should be revised to define adverse events and to clarify reporting time frames, and to establish clinical follow-up responsibilities after adverse events.

COMMITTEE'S STATEMENT OF TASK

In addition to implementing corrective actions in response to the OIG's recommendations, EPA sought independent expert advice from the National Academies of Sciences, Engineering, and Medicine to address scientific issues and provide guidance on the conduct of CHIE studies. The committee was asked to assess the utility of CHIE studies to inform and reduce uncertainties in setting air-pollution standards to protect public health and assess whether continuation of such studies is warranted. The committee also was asked to assess the potential health risks to test subjects who participated in recent studies of air pollutants at EPA's clinical research facility and comment on the degree of actual risk imposed by the exposures in those studies.

If the committee supports continued conduct of CHIE studies, it was asked to provide further guidance on methods for estimating levels of risk in CHIE studies. In addition, the committee was asked to provide advice on a template for characterizing reasonably foreseeable risks, which could be used in obtaining informed consent from potential study participants. (The committee's formal statement of task is presented in Box 1-2.)

TERMS FOR REFERRING TO INDIVIDUALS EXPOSED IN CHIE STUDIES

This report uses the terms "participant" and "subject," and occasionally "volunteer" or "healthy volunteer," to refer to persons who enroll in EPA's CHIE studies of environmental pollutants. Each term has assets and liabilities. "Subject" has been widely used for decades, and appears in most ethical codes and in the federal regulations. It correctly suggests that those who enroll are subjected to the requirements of a preapproved research protocol. Yet it can suggest passivity rather than active involvement. "Participant" has become more common in recent years both internationally and in the United States (NBAC, 2001). The National Bioethics Advisory Committee supported the use of the term "human participant" as a signal of respect for those who enroll in research and to emphasize that individuals should be active, not passive, in the decision to join research studies (NBAC, 2001). However, the participation of enrollees is quite limited; they have nothing to do with the design or conduct of the research. "Volunteer" or "healthy volunteer" implies that participation is a freely chosen decision, and that the research under consideration is not associated with medical care. Yet volunteers are paid for their participation. All three terms are of value, since each emphasizes an important feature of human-subjects research. Individuals involved in the EPA research described in this report volunteer to become participants and, in so doing, become subject to the requirements of the research protocol. Generally, this report considers these terms synonyms but sometimes uses them selectively, to emphasize a particular feature of enrollees.

[1]EPA's responses to OIG's recommendations are provided in EPA, unpublished material, April 27, 2015.

Controlled Human Inhalation-Exposure Studies at EPA

BOX 1-2 Committee's Statement of Task

An ad hoc committee will address scientific issues and provide guidance on the conduct of controlled human-exposure studies designed to inform policy decisions and set air-pollutant standards to protect public health. The committee will consider EPA's Office of Inspector General report titled Improvements to EPA Policies and Guidance Could Enhance Protection of Human Study Subjects, which recommends improvements to EPA's conduct of studies and outlines how the agency intends to address the recommendations. Relevant issues and questions for the committee to address include the following:

- EPA has performed controlled human-exposure studies to help understand exposure to and potential health effects of common air pollutants, such as particulate matter. To what extent have such studies been valuable to inform and reduce uncertainties in setting pollutant standards? Is it warranted to continue to conduct controlled human-exposure studies as part of EPA's larger research agenda for air pollutants?
- The committee will assess the potential health risks to test subjects who participated in recent studies of air pollutants at EPA's clinical research facility and comment on the degree of actual risk imposed by the exposures in those studies.
- If the committee supports continued conduct of human-exposure studies, it will provide further guidance in the following areas:
  - Methods for estimating levels of risk in controlled human-exposure studies, drawing from relevant approaches used in Phase I clinical drug trials, and
  - A template to characterize reasonably foreseeable risks, in terms of the nature, frequency, and magnitude of possible risks, which could be used in obtaining informed consent from potential study participants. The literature on "challenge studies" should be considered in the design of the template, if applicable.

ORGANIZATION OF THE REPORT

In Chapter 2, the committee provides the foundational context for the report in terms of language and concepts related to CHIE studies at EPA. That chapter also discusses the committee's approach used in carrying out its study charge. Chapter 3 discusses the scientific value of CHIE studies for EPA decision making concerning air quality management. Chapter 4 discusses the risks of clinical adverse events posed to CHIE study participants in past studies. In addressing whether the continued conduct of CHIE studies at EPA is warranted, Chapter 5 considers the level of risk posed to study participants and whether CHIE studies are capable of filling relevant knowledge gaps, at least in part. Chapter 6 discusses methods EPA could use for characterizing short- and long-term risks to CHIE study subjects in the future for the purposes of informing IRBs and potential participants. Chapter 7 addresses communication to potential study participants about informed consent.

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Foundational Aspects of Human-Subjects Research

INTRODUCTION

During its deliberations, the committee recognized a need to articulate key concepts and definitions over a range of disciplines as part of addressing its statement of task. This chapter is organized in sections that introduce the subject areas that comprise this report, which include air pollution health effects science, air pollution regulation, research involving human subjects, and research oversight. The committee's approach included the identification of key documents in each of the subject areas that are relevant to the statement of task.

Establishing a clear vocabulary and concepts is intended to set the stage for answering the questions "To what extent have controlled human inhalation exposure (CHIE) studies been valuable to inform and reduce uncertainties in setting pollutant standards?" (Chapter 3), "What are the risks of clinical adverse events posed to CHIE study participants in past studies?" (Chapter 4), "Is it warranted to continue to conduct CHIE studies as part of the U.S. Environmental Protection Agency's (EPA's) larger research agenda for air pollutants?" (Chapter 5), and "What methods should be used to characterize and communicate risks associated with participating in CHIE studies?" (Chapters 6 and 7). Below we introduce terms and topics that are presented in greater detail in this chapter and Chapter 3. The purpose here is to orient the reader to our methodologic approach, introduce topics and terms, and describe the organization of the report narrative.

The committee assessed the value of CHIE studies by considering the body of knowledge derived from those studies and the integration of that knowledge with the results of toxicologic and epidemiologic studies in the development of EPA's Integrated Science Assessments (ISAs)—documents which inform decision making concerning the National Ambient Air Quality Standards (NAAQS), as discussed below. The committee utilized the ISAs to assess how others had utilized CHIE study results and to assess the benefits they provide. Considerations of causality in epidemiology and public health developed by Sir Austin Bradford Hill (Hill, 1965), and as adapted by EPA for its ISAs, were used by the committee as an organizing framework for describing different dimensions of the values provided by CHIE studies (see Chapter 3).

To address whether it is warranted to continue to conduct CHIE studies as part of EPA's larger research agenda for air pollutants, the committee reviewed recent strategic plans of EPA's Office of Research and Development and identified some remaining knowledge gaps in air pollution health effects science (see Chapter 5) that influence the issues being discussed herein.

The committee took a more narrow approach in considering risks to test subjects and issues of informed consent. In this instance, the committee utilized the eight CHIE studies provided by EPA to illustrate current practices for articulating study objectives, rationale, and design. Committee members considered in detail the screening processes, inclusion/exclusion criteria, consent procedures, and characterizations of risks to study participants for the Institutional Review Board (IRB). In addition, the committee assessed the adverse events reported to the University of North Carolina IRB for all CHIE studies for the period from January 2009 to February 2015.The committee also considered the ethical analysis framework articulated in the 2004 National Research Council (NRC) report *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues* (2004a) in its review of these eight studies.

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The committee used requested documentation provided by EPA to develop an understanding of the structure of the CHIE study processes, including its multiple internal oversight steps and opportunities for external input. Through the information provided to the committee, EPA personnel demonstrated a detailed understanding of issues central to the committee's task. The committee found EPA's procedures to be consistent with, and indicative of, ethical approaches to human-subjects research, and that finding is consistent with those of EPA's Office of Inspector General's report (see Chapter 1).

The committee developed its report in consideration of themes that represent the continuing evolution of scientific knowledge, social and ethical thought, and public policy. The committee also was cognizant of the importance of harmonizing terminology, where possible, to facilitate communication across institutions and disciplines. In general, the committee adopted the language and conventions of human-subjects research and IRBs for describing biologic responses and adverse events. In contrast, the methods for characterizing risk of adverse events to study subjects draws upon language and conventions of epidemiology, toxicology, risk assessment, and communication sciences.

AIR POLLUTION HEALTH EFFECTS SCIENCE FOR MANAGING AIR QUALITY

EPA states that one of its purposes is to ensure that "all parts of society—communities, individuals, businesses, and state, local and tribal governments have access to accurate information sufficient to effectively participate in managing human health and environmental risks" (EPA 2017b). Accurate information about the impact of environmental pollutants on human health is thus a precious commodity, assembled through painstaking, multidisciplinary, iterative efforts. The committee was unanimous in its view of the importance of a robust body of valid scientific information that supports that purpose of EPA.

The committee's focus is on understanding the health impacts of inhalation exposures to pollutants in the outdoor ambient air in an individual's immediate surroundings, both when outdoors and when exposed to outdoor pollutants that have penetrated into indoor environments. There is no single approach, no simple oracle that provides this information. Originating in the congressional mandate of the Clean Air Act (CAA), there is a process through which scientists compile and interpret this scientific information on air pollution and health effects, which is then translated into primary (health-related) and secondary (welfare-related) NAAQS (see Table 2-1), which lead in turn to emission control strategies. Because the secondary standards do not focus on human health, they therefore are not within the committee's scope of work.

As mandated by the CAA, NAAQS have been established for each of the six current criteria pollutants and the NAAQS for each pollutant must be reviewed periodically using a well-defined process. Included in this process is the development by EPA's Office of Research and Development (ORD) of an ISA. The ISA is an extensive review and summary of peer-reviewed literature relevant to the NAAQS (see Chapter 3).

The composition of ambient air and the concentration of its specific constituents are determined by multiple natural and anthropogenic sources and factors, including emission sources related to mobile sources (such as vehicular traffic), area sources[1] (such as dry cleaning facilities), and larger stationary sources (such as electricity generation facilities). Each human individual has a personal exposure profile, determined in part by his or her regular activities. Decades of research, as summarized in the ISAs and other reports, show that to a remarkable degree, exposures to pollutants in the local outdoor air (or ambient air) influence a broad range of health outcomes (EPA, 2009, 2013; NRC, 2004b).

The scientific studies contributing to the knowledge of air pollution–related health effects, and hence establishing the basis for regulation, include toxicologic studies (using whole animals or cellular cultures), observational studies of health-related responses to exposures while humans are engaged in their regular activities, and CHIE short-term inhalation exposure studies of volunteer subjects to individu-

[1]According to EPA, "area" sources are those sources that emit less than 10 tons annually of a single hazardous air pollutant or less than 25 tons annually of a combination of hazardous air pollutants (EPA, 2016d).

al criteria pollutants or mixtures. Each study type has strengths and limitations, but together they can provide a basis for informing EPA decision making (Brown et al., 2007; EPA, 2009, 2013).

Observational studies, such as epidemiologic studies and panel studies, are used to identify associations between ambient pollution concentrations and adverse health effects by examining changes in various health end points, within an entire population or particular subgroups. These studies examine effects associated with short- or long-term exposures to pollutant mixtures, which are generally not controlled by the researcher. However, they are unlikely to elucidate biologic mechanisms or establish causal relationships.

TABLE 2-1 National Ambient Air Quality Standards for Six Criteria Pollutants (as of December 2016)

Pollutant	Primary/Secondary[a]	Averaging Time	Level (Concentration)	Form[b]
Carbon Monoxide (CO)	Primary	8 hours	9 ppm	Not to be exceeded more than once per year
		1 hour	35 ppm	
Lead (Pb)	Primary and secondary	Rolling 3-month average	0.15 μg/m^3[c]	Not to be exceeded
Nitrogen Dioxide (NO_2)	Primary	1 hour	100 ppb	98th percentile of 1-hour daily maximum concentrations, averaged over 3 years
	Primary and secondary	1 year	53 ppb[d]	Annual Mean
Ozone (O_3)	Primary and secondary	8 hours	0.070 ppm[e]	Annual fourth-highest daily maximum 8-hour concentration, averaged over 3 years
Particulate Matter (PM) $PM_{2.5}$	Primary	1 year	12.0 μg/m^3	Annual mean, averaged over 3 years
	Secondary	1 year	15.0 μg/m^3	Annual mean, averaged over 3 years
	Primary and secondary	24 hours	35 μg/m^3	98th percentile, averaged over 3 years
PM_{10}	Primary and secondary	24 hours	150 μg/m^3	Not to be exceeded more than once per year on average over 3 years
Sulfur Dioxide (SO_2)	Primary	1 hour	75 ppb[f]	99th percentile of 1-hour daily maximum concentrations, averaged over 3 years
	Secondary	3 hours	0.5 ppm	Not to be exceeded more than once per year

[a]The Clean Air Act requires EPA to set two types of NAAQS: primary NAAQS to protect public health, and secondary NAAQS to protect the public welfare from known and anticipated adverse effects (such as crop damage from pollutant exposure).

[b]The form defines the air quality statistic that is to be compared to the standard in determining whether an area attains the NAAQS.

[c]In areas designated nonattainment for the Pb standards prior to the promulgation of the current (2008) standards, and for which implementation plans to attain or maintain the current (2008) standards have not been submitted and approved, the previous standards (1.5 μg/m^3 as a calendar quarter average) also remain in effect.

[d]The level of the annual NO_2 standard is 0.053 ppm. It is shown here in terms of ppb for the purposes of clearer comparison to the 1-hour standard level.

[e]Final rule signed October 1, 2015, and effective December 28, 2015. The previous (2008) O_3 standards additionally remain in effect in some areas. Revocation of the previous (2008) O_3 standards and transitioning to the current (2015) standards will be addressed in the implementation rule for the current standards.

[f]The previous SO_2 standards (0.14 ppm 24-hour and 0.03 ppm annual) will additionally remain in effect in certain areas: (a) any area for which it is not yet 1 year since the effective date of designation under the current (2010) standards, and (b) any area for which implementation plans providing for attainment of the current (2010) standard have not been submitted and approved and which is designated nonattainment under the previous SO_2 standards or is not meeting the requirements of a SIP call under the previous SO_2 standards (40 CFR 50.4(3)). A SIP call is an EPA action requiring a state to resubmit all or part of its State Implementation Plan to demonstrate attainment of the required NAAQS.

SOURCE: Adapted from EPA (2016c).

Toxicologic studies of experimental exposures to whole animals provide the capability of assessing possible biologic mechanisms for a broad range of health end points in response to specific pollutants or ambient mixtures. In addition, toxicologic studies can provide relevant information on causality when the biologic responses observed are similar to responses expected in humans under ambient exposure conditions. However, uncertainty is introduced when results are extrapolated to humans. Results from *in vitro* studies using cell cultures that probe interactions of chemicals with cellular components could provide mechanistic information or support for results from whole-animal studies.

CHIE studies of human volunteers are used to help understand relationships between short-term exposure to a known concentration of a defined pollutant or pollutant mixture and an adverse health effect. They also can be used to evaluate the relevance of biologic mechanisms observed in animals, and they can provide information on the biologic plausibility of associations observed in epidemiologic studies. Controlled studies provide the potential for demonstrating causality for the conditions of the particular exposure protocol (for example, specific exposures and the health status of the study subjects). Although some CHIE studies have included health-compromised study subjects, such as those with certain respiratory or cardiovascular diseases, they are not likely to represent the most sensitive individuals in the population (as discussed in subsequent chapters).

Outdoor air quality is a societal resource, protected by the CAA and regulations imposed by EPA. The CAA requires EPA to periodically review the NAAQS for each of its criteria pollutants (specified at 5-year intervals, but often needing more time for completion of its review) and either retaining them or revising them in the light of new information. EPA is currently in the process of reviewing the 2009 NAAQS for particulate matter (PM) (EPA, 2009) in order to take into account the hundreds of peer-reviewed research and review papers that have been published since completion of the prior review. As noted in the chapters of this report that follow, many of these more recent publications confirm and extend the conclusion of the 2009 ISA that ambient air PM has been shown to influence a broad range of pulmonary and cardiovascular health outcomes.

The CAA also mandates that EPA establish and enforce National Emission Standards for Hazardous Air Pollutants (NESHAPs) to protect public health from exposures to known toxicants emitted from definable point sources. Each NESHAP establishment or revision is reviewed by the EPA Administrator following the finalization of the supporting scientific documents by the Environmental Health Committee of EPA's Science Advisory Board that met in public for its deliberations. The regulatory process for NESHAPs focuses on emissions, rather than ambient air concentrations. However, because the intent of NESHAPs is to protect public health, there is a need for a scientific base of knowledge about the human toxicity of these pollutants at ambient concentrations. Although CHIE studies are most often used to inform NAAQS decision making, they potentially could be used to inform NESHAPs decision making. CHIE studies involving NESHAP pollutants would be subject to the same considerations and reviews as those articulated for NAAQS pollutants.

One goal of our committee's report is to inform a national conversation about the role of CHIE studies in developing accurate air pollution health-effects information. This conversation, in turn, is part of an evolving and lively discussion about what constitutes ethical research, whether it involves humans or animals. The conversation is informed by social norms and sensibilities, by acknowledgment of past mistakes and transgressions, by divergent views about the risks and benefits of this type of research, and by discussions about what constitutes informed consent of human study subjects. In this chapter, we provide a framework for our deliberations, and reference documents that are benchmarks in this continuing conversation.

HUMAN-SUBJECTS RESEARCH

CHIE studies have been conducted for many decades within and outside of EPA to investigate relationships between air pollution exposure and human health. The 2008 ISA for sulfur oxides (EPA 2008) cites CHIE studies that were undertaken in the 1950s and 1960s to investigate effects of sulfur dioxide (SO_2) exposure on short-term lung function. For example, Amdur et al. (1953) and Frank et al. (1962)

were cited, among other studies, to have observed human respiratory effects at SO_2 exposure concentrations greater than 1 ppm, including increased respiration rates, decrements in peak flow, bronchoconstriction, and increased airway resistance. EPA has been conducting human experimental exposure studies since 1973. The agency has used results of CHIE studies of O_3 exposure and respiratory symptoms, conducted within and outside of EPA, to inform the O_3 NAAQS decisions in 1979, 1997, 2008, and 2015. In the 2000s, there was greater use of PM CHIE studies to investigate possible mechanisms of health effects observed in epidemiologic studies.[2]

As stated in Chapter 1, EPA asked the National Academies of Sciences, Engineering, and Medicine to assess whether it is warranted to continue to conduct CHIE studies as part of EPA's larger research agenda for community air pollutants and, if so, to recommend guidance to improve methods of characterizing risks to study subjects and improving informed consent. Underlying that task is the question of whether the CHIE studies meet the requirements for ethical human-subjects research and whether the data and scientific benefits derived from those studies can be ethically balanced against the risks posed to the human subjects who participate in the CHIE studies. This risk–benefit balance is imposed by the standards that govern human research in the United States: the Federal Policy for the Protection of Human Subjects, better known as the "Common Rule," which governs human research conducted by EPA as well as most other federal agencies. Therefore, the committee's task has not been to reinvent the ethical structure of the inquiry but rather to consider how that ethical structure should be interpreted in the context of CHIE studies, where the potential risks are borne by the subjects involved in the studies. Volunteers for CHIE studies routinely undergo physical exams to determine their eligibility to participate, and in the process might discover health information of value to them. Such information should not be considered a benefit of participation, because such health information is not the aim of CHIE studies and is fortuitous. Instead, the benefits are enjoyed by society and not by the individuals participating, except within their lives as members of society.

During its information-gathering session on August 24, 2016 (see Appendix B), the committee heard concerns that CHIE studies involving any exposures of study subjects to PM concentrations greater than the NAAQS concentration limit are unsafe and unethical. However, reliance only on a concentration for evaluations in this context is not consistent with the concept of the NAAQS. Each of the NAAQS is stated in terms of an averaging time and a statistical form as well as a concentration (level) (see Table 2-1). A NAAQS concentration for $PM_{2.5}$ averaged over 24 hours is permissible on 2% of days averaged over 3 years. The underlying rationale is based on the relationship between cumulative dosages delivered from short-term exposures (24 hours for PM) and acute responses, allowance for extreme meteorological conditions favoring a temporary buildup of pollution, and the margin-of-safety factor built into the setting of a NAAQS. Therefore, evaluation of CHIE studies with respect to the applicable NAAQS needs to consider the exposure concentration and duration. It is also important to consider the adequacy of the process used to screen the volunteer subjects for entry into the study and the medical monitoring of the subjects during the course of the inhalation exposures.

A Brief History of Progress and Ethical Lapses in Human Subjects Research

The history of progress in defining ethical research is summarized in Table 2-2. An accounting of unethical experiments of the past provides a sobering reminder of the paramount importance of careful attention to protocols and procedures, including consent by participants. The committee's detailed review of eight CHIE studies, including protocols, procedures, and consent forms, reflects a recognition of the importance of this attention.

Behind each of the milestones listed in Table 2-2 lies an event or a pattern of events that demonstrates the need for the current ethical oversight. Researchers forget these precipitating events at their peril. Both in terms of the safety and well-being of research participants, and also in terms of the social un-

[2]EPA presentation to the committee, June 1, 2015.

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derstanding and support for scientific research, it is important to remember the lapses that led to the current requirements for ethical research practices. For example, the Nuremberg Code was written in response to the atrocities perpetrated by the Nazi physicians during World War II (Nuremberg Military Tribunals, 1949). The code's emphasis on informed consent, avoiding harms to subjects, and the freedom of subjects to withdraw at any time speak to the absence of all three of these features in the Nazi experiments and provides a basis for preventing unethical experiments in the future. The Declaration of Geneva, dating from 1949, recognizes that researchers are often physicians; it marks the start of a much deeper understanding of research ethics as having different and more stringent ethical requirements than those reflected in ordinary medical ethics codes. Likewise, development of the Belmont Report and U.S. Department of Health and Human Services regulations during the 1970s were a direct result of the revelations of deceit, abuse, and racism in the Tuskegee Syphilis Studies conducted over a 40-year period by the U.S. Public Health Service. After many decades of learning reactively from missteps in research, it is important to be proactive, anticipating problems and providing ethical safeguards against them. In preparing this report, the committee sought to build upon this history of progress by providing more refined and particular ethical guidance for the kind of research done by the EPA, and others, in CHIE studies and other research that enrolls human volunteers. In so doing, the committee hopes to advance the societal collective approach to human-subjects research.

As presented in Table 2-2, guidance from the Food and Drug Administration (FDA) indicates that clinical trials should be registered on the U.S. National Institutes of Health website: www.Clinical Trials.gov. Editorial policies for scientific journals generally require that this be done in order for study results to be considered for publication. The committee supports the practice of registering CHIE studies on this website. Although many clinical trials have a therapeutic end point as their primary goal, the committee considers the practice of registering CHIE studies on the website as a way to encourage the harmonization of human-subjects protection practices. In addition, registering CHIE studies will help make other investigators aware of this proposed research, potentially preventing duplication and providing opportunities for studies to build upon one other.

Requirements for Ethical Research

The committee reviewed the basis for prior deliberations which concluded that CHIE studies were examples of ethical research. Table 2-3 provides seven requirements for determining whether a human research trial is ethical and is particularly valuable because it presents a coherent principle-based framework for evaluating the ethics of clinical research projects. This framework distills the requirements and insights of a large range of both historical and contemporary ethical statements from U.S. and international sources. The seven requirements are social or scientific value, scientific validity, fair subject selection, favorable risk–benefit ratio, independent review, informed consent, and respect for potential and enrolled subjects. None of the requirements can stand alone as a sufficient justification for a human-subjects research project. While not all are necessary to all forms of research, for example, some emergency research has been exempted from an informed consent requirement, all seven are ethically necessary for EPA's CHIE studies. Table 2-3 provides additional details, including the ethical values that justify each of the requirements. For example, the social or scientific value of a project is required by considerations of distributive justice (not wasting the scarce resources available for research) and by nonexploitation (not placing human subjects at risk for research that has no scientific or social value). Table 2-3 also indicates the kind of expertise needed to make an evaluation that each of the seven requirements is satisfied. For example, for the informed-consent requirement, expertise in scientific knowledge about the purpose of the trial and the risks and benefits to which participants will be exposed is essential. Equally important, legal and ethical expertise is needed to ensure best practices in the consent process, described in Chapter 7 as disclosing information, encouraging deliberation, and enabling authentic decision making. Table 2-3 provides the ethical foundation on which the deliberations and recommendations of this report are built. Further discussion of the ethical meanings and uses of the concepts of *informed consent*, *risk*, and *benefit* are provided in Chapter 7, along with recommendations about communication of these concepts to research participants.

TABLE 2-2 Selected Laws, Ethical Codes, and Other Milestones Related to the Protection of Human Subjects

Milestone	Year	Description
U.S. Food and Drug Act	1938	Requires that drugs be shown to be safe before marketing, which leads to the need for human trials.
Nuremberg Code	1947	• The voluntary consent of the human subject is absolutely essential. • The experiment should aim at positive results for society that cannot be procured in some other way. • The experiment should be justified by a sound study design, results from animal studies or knowledge of the natural history of the disease, and the anticipated results. • The experiment should be so conducted as to avoid all unnecessary physical or mental suffering or injury. • No experiment should be conducted when there is any reason to believe that death or disabling injury will result. • The risks of the experiment should be never exceed the expected humanitarian benefits. • Preparations and facilities must be provided that adequately protect the subjects against even the remote possibility of injury, disability or death. • Only scientifically qualified persons may conduct the experiment, and the highest degree of skill and care should be required of all involved. • The human subjects should be free to bring their participation to an end when they judge their physical or mental health would make continuation impossible. • The scientist in charge must be prepared to terminate the experiment when there is probably cause to believe that continuation is likely to result in injury, disability or death to the experimental subject.
International Code of Medical Ethics of the World Medical Association, including the Declaration of Geneva	1949	• A physician shall always bear in mind the obligation of preserving human life. • The health of the patient shall be the physician's first consideration. • A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient.
Helsinki Declaration	1964	• Clinical research should be based on animal and laboratory experiments. • Clinical research should be conducted and supervised only by qualified medical workers. • Clinical research should be preceded by a careful assessment of risks and benefits to the patient. • Human beings should be fully informed and must freely consent to the research. • Responsibility for the human subject must always rest with a medically qualified person, and never with the subject. • Results of experiments that do not comply with ethical guidelines should not be accepted for publication. • Special care must be taken with informed consent of minors. • Consideration of the welfare of animal subjects and the environment is also mentioned.
U.S. Surgeon General policy statement	1966	• All human subject research requires independent prior review. • Origin of Institutional Review Boards (IRBs).
Regulations for the Protection of Human Subjects of Biomedical and Behavioral Research (45 CFR 46)	1974	IRB procedures established.

(Continued)

TABLE 2-2 Continued

Milestone	Year	Description
Belmont Report (Report on Ethical Principles and Guidelines for the Protection of Human Subjects of Research) (NCPHSBBR, 1979)	1979	• Principle of Respect for Persons—the obligation to treat subjects as autonomous agents and to protect persons with diminished autonomy. • Principle of Beneficence—the obligation not to harm subjects and to maximize possible benefits to subjects and minimize potential harms. • Principle of Justice—the obligation to distribute fairly both the benefits and burdens of research.
President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research	1980-1983	Recommended that all federal agencies adopt the human subject regulations of the Department of Health and Human Services (HHS, formerly DHEW).
Common Federal Policy for the Protection of Human Subjects ("Common Rule") [10 CFR 745]	1991	Sixteen agencies adopt the regulations of 45 CFR 46 subpart A. Subparts B, C, and D adopted by many agencies.
FDA Guidance for Sponsors, Investigators and IRBs (FDA, 2012)	2012	For applicable clinical trials initiated on or after March 7, 2012, informed-consent documents must be in compliance with the new requirement in 21 CFR § 50.25(c) and include a specific statement that refers to the trial's description on www.ClinicalTrials.gov.

Source: Adapted from Sparks 2002.

TABLE 2-3 Seven Requirements for Determining Whether a Research Trial Is Ethical[a]

Requirement	Explanation	Justifying Ethical Values	Expertise for Evaluation
Social or scientific value	Evaluation of a treatment, intervention, or theory that will improve health and well-being or increase knowledge	Scarce resources and nonexploitation	Scientific knowledge; citizen's understanding of social priorities
Scientific validity	Use of accepted scientific principles and methods, including statistical techniques, to produce reliable and valid data	Scarce resources and nonexploitation	Scientific and statistical knowledge; knowledge of condition and population to assess feasibility
Fair subject selection	Selection of subjects so that stigmatized and vulnerable individuals are not targeted for risky research and the rich and socially powerful not favored for potentially beneficial research	Justice	Scientific knowledge; ethical and legal knowledge
Favorable risk–benefit ratio	Minimization of risks; enhancement of potential benefits; risks to the subject are proportionate to the benefits to the subject and society	Nonmaleficence, beneficence, and nonexploitation	Scientific knowledge; citizen's understanding of social values
Independent review	Review of the design of the research trial, its proposed subject population, and risk–benefit ratio by individuals unaffiliated with the research	Public accountability; minimizing influence of potential conflicts of interest	Intellectual, financial, and otherwise independent researchers; scientific and ethical knowledge
Informed consent	Provision of information to subjects about purpose of the research, its procedures, potential risks, benefits, and alternatives, so that the individual understands this information and can make a voluntary decision whether to enroll and continue to participate. Subjects have the right to withdraw at any time.	Respect for subject autonomy	Scientific knowledge; ethical and legal knowledge
Respect for potential and enrolled subjects	Respect for subjects by (1) permitting withdrawal from the research, (2) protecting privacy through confidentiality, (3) informing subjects of newly discovered risks or benefits, (4) informing subjects of results of clinical research, and (5) maintaining welfare of subjects	Respect for subject autonomy and welfare	Scientific knowledge; ethical and legal knowledge; knowledge of particular subject population

[a]Ethical requirements are listed in chronological order from conception of research to its formulation and implementation.
Source: Emanuel et al. (2000). Reprinted with permission; copyright 2000, *Journal of the American Medical Association*.

RISK–BENEFIT FRAMEWORK

As noted earlier in this chapter, ethical clinical research must be bounded by a favorable risk–benefit ratio. Indeed, not only must the ratio be favorable, but IRBs—and researchers—are required to ensure that risks are minimized to the extent possible even within a favorable risk–benefit ratio. The 2004 NRC re-

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port on intentional human dosing, introduced in Chapter 1, presented a framework for comparing risks and societal benefits for EPA studies that involve deliberate exposures of human subjects to specific chemicals and/or other potentially toxic materials, such as pollutant mixtures. In that 2004 report, the authoring committee determined that it is ethically acceptable to involve volunteers in studies that expose them to somewhat higher risks than studies that pose no identifiable risks or for which there is a reasonable certainty of no harm, if the studies have the potential of providing a clear health or environmental benefit to society and the studies are expected to cause no lasting harms to study participants (NRC, 2004a, p. 105). It provided examples of two extremes, one involving a type of study that was almost certainly acceptable and the other involving a study that was almost certainly unacceptable (NRC, 2004a, p. 107). The former involved a study where the exposure was designed to investigate pharmacokinetic information, including absorption of the chemical and the subsequent metabolism of the chemical, where the exposure has no known biologic effect on the participant. The latter involved a study in a medically vulnerable population with the potential for lasting adverse effects. While these examples present clear boundaries for what would be ethical and unethical research, the 2004 report notes that cases between these two extremes are more difficult to evaluate. Such cases might require sophisticated risk assessments and understanding about the short- and long-term effects of the chemicals involved; this might be beyond the capability of a typical IRB.

Since the publication of the 2004 report, there has been considerable scholarly attention to the risk–benefit ratio as part of the broader ethical framework for biomedical research. In 2011, Annette Rid and David Wendler offered a comprehensive step-by-step method for making risk–benefit evaluations (Rid and Wendler, 2011). This system is not a substitute for the judgment that is required by such an evaluation, but it ensures that appropriate attention is given to all facets of the evaluation.

- Step 1 requires that the proposed study achieve a minimum level of social value. Rather than doing that analysis after determining risks, Rid and Wendler believe that analyzing the expected value is an essential first step that focuses the research more appropriately. In the context of the CHIE studies, this means that the studies are expected to provide information for regulatory decision making that cannot be obtained by other means.
- Step 2 is to identify the research interventions and to ensure the safety of those interventions. Such interventions are expected to be focused on the question posed by the study and aligned with the social values identified in Step 1. In addition, they are expected to yield information likely to be important and nonduplicative. It may require alternatives to be considered to enhance safety.
- Step 3 is to evaluate and reduce or minimize any risks posed by the study. The first element of this step of course is to identify any potential risks, and we discuss this process in the next section on foreseeable risks. Risks are characterized by the probability, magnitude, timing of the onset, and duration of the potential harm. This step also requires another look at alternative procedures that might mitigate risks. Changes to inclusion and exclusion criteria might also mitigate risks. Here Rid and Wendler introduce an important distinction: the purpose of such a risk–benefit evaluation is "not to protect participants from risks … but *to reduce the extent to which participants experience harm from participating in a research study*" (p. 149).
- Step 4 is to evaluate and enhance the potential societal benefits for participants. In the CHIE studies, there are no anticipated medical benefits for participants. (See Chapter 7 for a discussion of other potential benefits.)
- Step 5 is to evaluate the extent to which potential clinical benefits might offset the risks of undergoing the intervention. (However, this step is not applicable to studies that do not offer clinical benefit and therefore it is not applicable to CHIE studies.)
- Step 6 is to evaluate the extent to which the net risks of some of these interventions might be justified by the potential clinical benefits of other interventions included in the same study. (Like Step 5, this step is not applicable to CHIE studies.)

- Step 7, the final step, determines whether the net risks are justified by the study's social value. Because of the iterative process of the evaluation, this step might provide a far more thorough analysis of the question than would occur if done less systematically.

One controversial issue that has not been resolved by bioethicists is whether there is an upper limit on how much risk can be tolerated in a study that may have very high social value but provide no direct benefits to participants. The Common Rule does not place any limits on such risks so long as the risk–benefit ratio is favorable. The Nuremberg Code tentatively endorses research that might result in death or disability, but only in those rare cases in which physician-researchers are also the research subjects. In its other provisions, the Nuremberg Code is lucid in its expressed opposition to research that incurs such serious risks. Some people argue that if informed consent is appropriately done, there should be no upper limit in a study involving competent adults. Others have less faith in the informed-consent process and prefer a more paternalistic approach. Resnick (2012) suggests that studies that pose more than a 1% risk of serious harm (defined as death, permanent disability, or severe illness or injury) should not ordinarily be allowed, absent a compelling public health interest and, even then, the acceptable risk should not exceed slightly more than 1%. Although the committee believes that Resnick's framework could have value by providing a level of acceptable risk, the framework presupposes that risks to CHIE study subjects can be quantified in a reliable manner. As discussed in Chapter 6, the committee recommends an alternative approach to characterizing risk.

Determining Reasonably Foreseeable Risk

The Common Rule requires that studies involving human-subjects research minimize risks to the extent possible, pose risks that are reasonable in relation to the benefits presumed (in the case of EPA CHIE studies, the knowledge expected to be gained that could inform EPA exposure standards), and that risks are appropriately communicated to the subjects. The statement of task for this committee specifically asks it to provide "a template to characterize reasonably foreseeable risks, in terms of the nature, frequency, and magnitude of possible risks, which could be used in obtaining informed consent from potential study participants." Of course, before the committee can consider how such risks should be communicated, it is important to consider how the potential clinical adverse effects of concern can be identified and characterized.

The term "reasonably foreseeable risks" appears in the Common Rule only as a requirement for informed consent, but it is implicit in the other requirements in the Common Rule for risk determination and assessment. The term is not defined in the rule, nor have any of the agencies adhering to the Common Rule attempted to define the term despite the fact that the issue has garnered considerable scholarly attention in recent years. Moreover, HHS issued an update to the Common Rule on January 19, 2017 (82 Fed. Reg. 7149 [2017]) but it does not deal directly with the issue. In fact, the OIG recommendation to EPA in the context of CHIE studies is but one of several recent calls for better guidance on this issue. For example, the HHS Secretary's Advisory Committee on Human Research Protections called on FDA to provide a better definition for studies involving FDA-regulated therapies. HHS has issued a draft guidance document designed to help researchers in studies evaluating standard of care treatments to present the reasonably foreseeable risks in the informed consent (OHRP, 2014). While that guidance is not directly applicable to CHIE studies, its logic can be extrapolated to them. It is beyond the scope of the committee's report to provide a complete framework for all studies covered by the Common Rule, but we attempt to provide such a framework for EPA's CHIE studies.

The term "reasonably foreseeable risks" as used in the regulations probably has its origins in legal tort law. As EPA notes, a general legal definition of the term is "a danger which a reasonable person should anticipate as the result from his/her actions" (Hill and Hill, 2014, p. 10). In negligence law, if one knows, or should know, of such a danger, one has a duty to avoid or mitigate that danger. Unfortunately, legal scholars have had as much trouble with the term as biomedical researchers, finding it vague and in-

determinate. Two prominent legal scholars note that "in one sense, everything is foreseeable, in another nothing" (Hart and Honore, 1985).

Nonetheless, some aspects of the term can be gleaned from its legal origins. First, it is not a strict liability standard. It does not require researchers to anticipate any hypothetical risk, no matter how remote, that might occur. On the other hand, as an objective standard, it requires researchers to undertake reasonable inquiry to determine what risks may exist. When Ellen Roche, a healthy volunteer, died during a study that sought to understand the pathophysiology of asthma using inhaled hexamethonium at Johns Hopkins University, the federal Office of Human Research Protection (OHRP) suggested that the researchers had failed to find earlier results of research in which subjects had experienced adverse events that exceeded those expected (Kennedy 2001). Although hexamethonium had not been used clinically for some decades, there was literature from the 1950s and 1960s that indicated adverse pulmonary reactions to the drug. Thus, determining "reasonably foreseeable risks" requires a thorough examination of existing literature and all available data to determine any potential adverse consequences.

In 1996, Hoiyan (Nicole) Wan died from a lethal dose of lidocaine that she received while participating in a M.I.T. sponsored medical research project at the University of Rochester Medical Center to investigate if the mutations in the bronchial cells of nonsmokers' lungs are principally caused by exposure to airborne pollutants. The lung cells were obtained using bronchoscopy, a procedure that involves the insertion of a flexible tube to gather lung cells for analysis. Lidocaine, an anesthetic, was given to make the study subject more comfortable during the procedure. The New York State Department of Health found that the University of Rochester had violated its own guidelines by increasing the dose of the anesthetic (Rosenthal 1996). This case is an example of how procedures and medications used in the procedures pose risks, and therefore need meticulous control and checks.

In its draft guidance, Disclosing Reasonably Foreseeable Risks in Research Evaluating Standards of Care, the Office for Human Research Protections (OHRP) states the following:

> The term "risk" refers to the likelihood that research harms or discomforts will occur, and to the nature and magnitude of those harms or discomforts. The risks of research in a study include those risks of therapies that some participating subjects would face that are or could be different from the risks of therapies they would have faced without participating in the research study. (OHRP, 2014)

In the context of CHIE studies, the sense of that guidance can be extrapolated to mean risks posed to a participant might be different than risks posed during that person's ordinary daily life. We agree with the approach taken by EPA in adopting the formulation posed by Resnik (2013): "A risk is reasonably foreseeable if we have some *credible evidence* to expect that it [a potential harm] may occur" (italics supplied). It is important to note that the formulation means there is an "expectation" that the harm *may* occur. That presents a more stringent criterion than for a hypothetical risk.[3] Because CHIE studies involve volunteers who will derive no therapeutic benefit from participation in the research, it is tempting to compare this criterion to "first in human studies" such as Phase 1 drug studies or even studies such as the hexamethonium study described above. But that is generally not an apt comparison because, unlike Phase 1 drug studies, or a study using a relatively novel agent as the challenge, EPA has a great deal of data about the various pollutants' biologic effects gleaned from epidemiologic studies, animal studies, and previous CHIE studies. The first step in determining "reasonably foreseeable risks" in the controlled-exposure studies is therefore to thoroughly study those data.

Observation of the Common Rule in the Development of EPA CHIE Studies

In this section the committee describes EPA's development and conduct of CHIE studies with respect to the Common Rule. The purpose of this section is to orient the reader to the terminology and us-

[3]EPA's responses to OIG's recommendations, page 10 (EPA, unpublished material, April 27, 2015).

ages of the review and consent process. The presentation uses an adapted version of the Rid and Wendler risk-benefit framework described above. As noted previously, two of the steps in that framework are inapplicable to studies that do not offer a medical benefit and are therefore inapplicable to the CHIE studies.

Step 1: Determine If the Proposed Study Is Designed to Achieve a Minimum Level of Social Value

The study designs are developed by a qualified Principal Investigator (PI) and undergo in-house review by other EPA staff to determine the extent to which the expected results of the study would support the review of NAAQS or other air quality–related decisions. The proposed study is reviewed by the branch chief who has a detailed knowledge of EPA ORD's strategic plans and can assess the study's expected value to society. Additional reviews by agency statisticians and controlled-exposure researchers, and outside experts also focus on the value of the study.

Step 2: Identify the Research Interventions and Ensure the Safety of Those Interventions

This step is completed through the submission of an application to the IRB. The University of North Carolina (UNC) Institutional Review Board is the governing IRB for CHIE studies which are conducted by EPA at its own Human Studies Facility on the campus of the University of North Carolina at Chapel Hill. IRBs at UNC are overseen by the director of the university's Office of Human Research Ethics. That person reports to the university's Vice Chancellor for Research, who is the authorized institutional official for UNC.

The IRB process has evolved over the 50 years since it was first created, with definitions that guide the review and reporting process. For the past 50 years, submission of an application for IRB review has been required when an activity includes both research and human subjects. Thus all CHIE studies are required to undergo IRB review and to receive approval prior to conducting the studies.

IRBs use the terms "minimal risk" and "more than minimal risk" within a triage mechanism to organize their review process, but use of those terms does not conduct quantitative risk assessments. IRBs assign submitted protocols for an expedited review if they are considered to pose minimal risk to study subjects. They assign a submitted protocol for full review if it is considered to pose "more than minimal risk." According to the Code of Federal Regulations (CFR) 46.102(i), minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.

The study protocols provided to the IRB for review will specify study-related risks posed by the controlled exposure ("exposure-related risks") and those posed by the procedures to be used to measure the selected health-related end points being measured ("assessment-related risks" or "procedure-related risks"). The protocol provides inclusion and exclusion criteria for participant recruitment, and specifies how these criteria will be assessed. The committee focused on factors that could alter participant susceptibility to study-related risks, such as preexisting medical conditions.

The IRB process provides an opportunity to demonstrate that the research methods focus on the question posed by the study, align with the social values identified in Step 1, and are nonduplicative. The IRB application provides the opportunity to discuss alternatives to be considered to enhance safety. A detailed study protocol that is submitted in addition to the IRB application enhances the transparency of this step of the process.

Step 3: Evaluate and Minimize Study-Related Risks to Participants

This step of the Rid and Wendler framework specifies the need to evaluate and minimize study-related risks. The committee recognizes that there are many dimensions to this step, as discussed below.

Controlled Human Inhalation-Exposure Studies at EPA

The ultimate ethical responsibility for all phases of the study lies with the PI. EPA has responsibility for oversight, and the IRB of record has the additional responsibility for monitoring the progress of the study and withdrawing its approval, if indicated. EPA has the responsibility for reporting serious adverse events, or any serious protocol noncompliance, to the reviewing IRB and to federal officials, should such occur. Additionally, prior to proposing the study, and while the study is ongoing, the PI is required to consider prior human, animal, and other biologic effects data that are available, to ensure that study-related risks to the study subjects are outweighed by the utility of the study results for informing air-quality management decisions. As mentioned previously, there is no medical benefit to the study subjects. With the foregoing in mind, Chapter 3 considers the value of the CHIE studies for informing EPA decision making.

Each proposed CHIE study is reviewed by two external experts to evaluate aspects of safety of study subjects, scientific rigor, and adherence to ethical principles (Personal communication, T. Schonfeld, EPA, July 2, 2015). CHIE studies involve placing study subjects in specially designed exposure rooms, where controlled concentrations of air pollutants are introduced and monitored. The subjects are enrolled after it is determined, through EPA's preexposure health evaluations, that there is no reason to believe that their participation in the study might lead to a clinically adverse effect.

The committee strongly supports the CHIE study practice of using a medical assessment to ascertain health status. The assessment protocol that is developed for a particular CHIE study is approved by the relevant IRB. Elements of the assessment include questions posed to the subject about the presence of medical conditions previously diagnosed by a physician, a physical examination, and selective testing. The medical assessments of study subjects might discover health information of value to them.

The health status of subjects is monitored throughout, shortly before and immediately after the controlled exposures, and again about 24 hours later. If, during the study, there is any evidence that an individual is being or has been harmed by the study, the person is referred for medical observation (or treatment). An individual can participate as a subject in up to six studies per year, provided that it is determined such enrollment will not be harmful to that individual.

Step 4: Evaluate and Enhance the Potential Benefits for Participants

As previously stated, there are no medical benefits for participating in CHIE studies. As discussed in Chapter 7 there are societal benefits and several types of personal benefits.

Step 5: Compare Risks and Societal Benefits

The final step of the Rid–Wendler framework asks whether the net risks are justified by the study's social value. IRB approval means that the net risks have been determined to be justified by the study's social value.

Terminology and Usages

Dimensions of Risk

We use the term "exposure-related risks" to refer to risks related to inhalation of the pollutants, and "procedure-related risks" to refer to risks related to measurement of study end points. In general, our primary focus is on exposure-related risks. Potential adverse outcomes associated with experimental procedures used during a CHIE study (for example, bronchoscopy) typically are well characterized through extensive experience in many kinds of clinical studies, and this information could be directly communicated to the IRB and the participants as part of communicating the risks associated with the conduct of the CHIE study.

Temporal Dimensions of Exposure-Related Risk

Focusing on exposure-related risks, the committee distinguished between risks of clinically adverse effects that might occur over the short term (for example, within 1 or 2 days) and risks of chronic conditions (such as cancer) that might develop over the long term. There is no bright line that separates short-term from long-term risks. Clinical practitioners stratify common health conditions based on symptom duration. For example, the National Institutes of Health (NIH) considers a cough to be acute if it lasts less than 3 weeks, subacute if it lasts 3-8 weeks, and chronic if it last longer than 8 weeks (NIH, 2010). L*ow back pain* is considered acute if it lasts less than 6 weeks, sub*chronic if it lasts* 6-12 weeks, and *chronic if it lasts* over 12 weeks (Chou, 2014). (See Chapter 4 for a discussion of temporal dimensions of CHIE-study exposure-related risk.)

Adverse and Serious Adverse Events

There are many acceptable definitions of adverse events. The committee has adopted the definition provided by HHS guidance (OHRP 2007) designed for adverse event reporting. The definition also is used by the IRBs at UNC Chapel Hill (UNC, 2014). An adverse event is

> Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

The committee also adopts the definition of "serious adverse event" that has been provided by the same HHS guidance:

> Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:
> 1. results in death;
> 2. is life threatening (places the subject at immediate risk of death from the event as it occurred);
> 3. requires inpatient hospitalization or prolongation of existing hospitalization;
> 4. results in a persistent or significant disability/incapacity;
> 5. results in a congenital anomaly/birth defect; or
> 6. any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).
>
> That definition is consistent with the one provided by UNC (2014): a "Serious Adverse Event (SAE) is one which is fatal or life threatening; results in significant or persistent disability; requires or prolongs hospitalization; results in a congenital anomaly/birth defect; or represents other significant hazards or potentially serious harm to research subjects or others."

Note that the duration or persistence of a biologic response is an important consideration in determining whether an adverse event is serious. The definition of a serious adverse event calls for a judgment as to whether an effect results in "a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions." For example, a cough lasting only 1 or 2 days after participation in a CHIE study would likely be considered an adverse event, while a cough that began soon after participation in a CHIE study and lasted many months would likely be a serious adverse event.

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Informed Consent

For an individual considering participation in a CHIE, the study is presented during an informed-consent process in which risk communication and risk perception play important roles. Informed consent is a process with three sequential elements, described in detail in Chapter 7. Information about a research study is presented to a potential participant in a disclosure process, the potential participant considers the information in a deliberative process, and the participant finally makes a decision to participate or not to participate.

Risk Perception

Risk perception is a subjective assessment resulting from a person's beliefs regarding the probability of a potential hazardous event or activity and how it will affect him or her. Individual philosophies, principles, and past experiences can shape one's beliefs about perceived risk. The severity of the risk and the overall public opinion of the risk can also affect individual risk perceptions (Beecher et al., 2005; Slovic, 1987).

Risk Communication

Risk communication is "any purposeful exchange of information about health or environmental risks between interested parties" (Covello et al., 1987). This information incorporates understanding, ideas, and actions as they relate to risks (Anderson and Iltis, 2008).

Exposure Comparators

The use of exposure comparators involves comparing experimental exposure concentrations and durations with ambient concentrations of similar magnitude and duration experienced by a population in everyday life at a certain location. That information is provided to individuals or IRBs for the purpose of enhancing their deliberation about the risks to participants involved in a CHIE study (see Chapter 6).

As IRBs are "consumers" of risk information, their deliberations are likely to be influenced by the individual risk perceptions of its members and of the board. The use of standard terminology for reporting adverse events to the IRB is intended to provide a common language to facilitate the IRB's work in balancing risks and benefits.

3

Value of Controlled Human Inhalation Exposure Studies

INTRODUCTION

The committee's assessment of the value of controlled human inhalation exposure (CHIE) studies centered on their contributions to the U.S. Environmental Protection Agency (EPA) regulatory decision-making process, especially with respect to promulgating air-quality standards. As discussed in Chapter 2, a key way to understand the value of CHIE studies in this process is by considering EPA's Integrated Science Assessments (ISAs). The ISAs are extensive reviews of policy-relevant science and consensus documents and are foundational to the process of reviewing the National Ambient Air Quality Standards (NAAQS) for the criteria pollutants.[1] (The NAAQS process is illustrated in Figure 3-1.) ISA drafts are reviewed by the Clean Air Scientific Advisory Committee and the public (EPA, 2015a). The use of ISAs is one of the ways the agency provides "access to accurate information sufficient to effectively participate in managing human health and environmental risks" (EPA 2017b). The regulation and control of the six current criteria air pollutants are considered to have broad public health importance because of the pollutants' anthropogenic origins and widespread distribution to many areas of the country.

Instead of assessing all of the contributions of CHIE studies to the NAAQS decision making for various criteria pollutants, the committee focused on their contributions to the ISAs for the NAAQS for ozone (O_3) and airborne particulate matter (PM). However, the committee's framework for evaluation is relevant to other criteria pollutants as well.

The CHIE studies carried out at EPA's Human Studies Facility during the past several years have focused mainly on O_3 and PM. (See Table C-1 in Appendix C.) They represent a contrast in composition complexity and variability (as discussed in this chapter). O_3 is a simple, single molecule, which is used as the indicator pollutant for the complex mixture of photochemical oxidants in ambient air. PM_{10} refers to particles with an aerodynamic diameter less than or equal to 10 μm. $PM_{2.5}$ refers to particles with an aerodynamic diameter less than or equal to 2.5 μm. Historically, EPA began by monitoring total suspended particulate matter, and then changed the indicator entity to PM_{10} in the 1997 PM NAAQS, but over recent decades the agency has focused more on $PM_{2.5}$ monitoring.

Table 3-1 lists the three NAAQS reviews conducted by EPA for O_3 and PM from 1996 to 2015. The most recent evaluation was reported in the *Integrated Science Assessment of Ozone and Related Photochemical Oxidants* (EPA, 2013). That document informed the review of the O_3 NAAQS completed in 2015.

In addition to informing the ISAs, CHIE studies of particles from specific sources (for example, diesel-engine exhaust particles and wood smoke particles) augment the scientific knowledge base for EPA's decision making concerning regulatory approaches that focus on source emissions (such as EPA's National Emission Standards for Hazardous Air Pollutants [NESHAP] for Stationary Reciprocating Internal Combustion Engines) (40 CFR Part 63, Subpart ZZZZ).

[1]The term "criteria pollutants" derives from the requirement in the Clean Air Act that EPA establish the scientific criteria for regulation by describing the characteristics and evidence of health and welfare effects of these pollutants.

Controlled Human Inhalation-Exposure Studies at EPA

A FRAMEWORK FOR EVALUATING CHIE STDUIES

The Hill aspects of causality in epidemiology and public health (Hill, 1965) have been used as an approach for assessment of the adequacy of evidence of a causal relationship between exposure to a hazardous agent and a possible health consequence (IOM, 2014). EPA adapted the Hill aspects for consideration of evidence in its ISAs. The considerations used by EPA include specificity of the association between an exposure and an observed response, temporality between the occurrence of an exposure and an observed association, a biologic gradient in the relationship between exposures and responses (such as increasing effects associated with greater exposures), plausibility of a proposed biologic mechanism for the occurrence of an effect, consistency (or reproducibility) of results across independent studies, coherence of observed outcomes across different fields of study or study designs, and experimental results indicating that a change in exposure can cause a change in a response (EPA, 2015a).

The EPA-adapted Hill aspects provided the committee with a framework for assessing the value of CHIE study results to inform EPA's regulatory decision making and for identifying the kinds of useful information CHIE studies can provide. Here we provide an overview of the values of CHIE studies according to those considerations. Details are provided later in the chapter.

Specificity and Experimental Findings: CHIE studies enable investigators to separate the effects of exposure to individual criteria pollutants, or specific groups of criteria pollutants, from effects associated with exposures to ambient complex mixtures that are observed in epidemiologic studies. The experimental study design of CHIE studies enables formal tests of hypotheses and more unambiguous assessments of short-term exposure–response relationships for specific laboratory-generated pollutants or mixtures. This allows EPA to focus on the causative agents in complex mixtures responsible for the observed health effects.

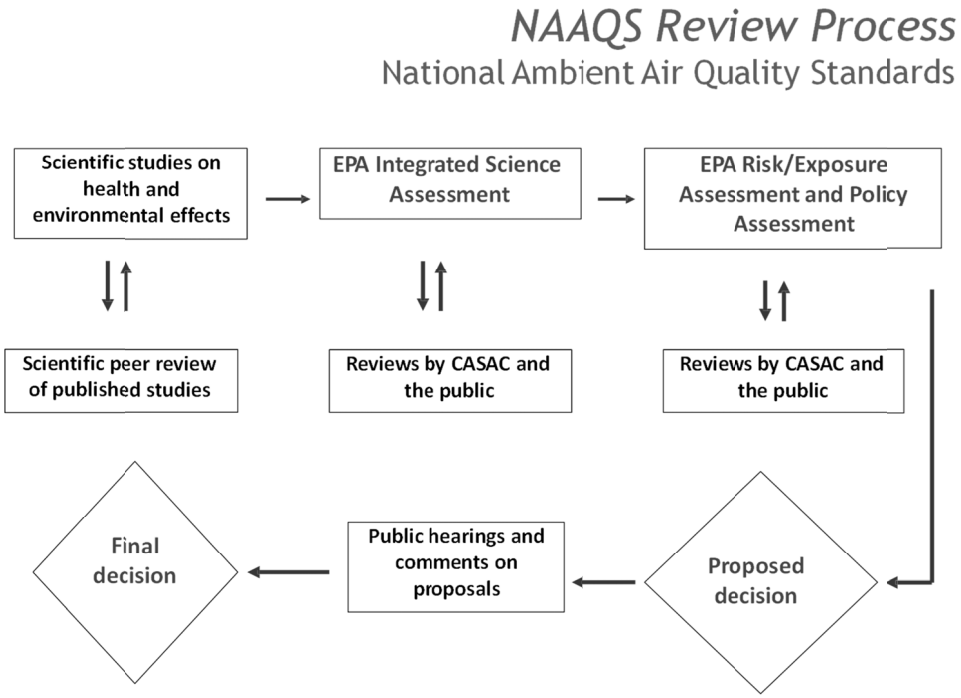

FIGURE 3-1 NAAQS review process. Source: EPA presentation to the committee on June 1, 2015.

TABLE 3-1 EPA's Reviews of Relevant Scientific Information and Revised NAAQS for O_3 and PM from 1996 to 2015

Review of Latest Relevant Scientific Information[a]	Year Finalized	Year NAAQS Revisions Completed	Indicator	Averaging Time	Level (Concentration) for Primary Standard[b]	Form[c]
Air Quality Criteria for Ozone and Related Photochemical Oxidants	1996	1997	O_3	8-hour	0.08 ppm	Annual fourth-highest daily maximum 8-hour concentration, averaged over 3 years
Air Quality Criteria for Ozone and Related Photochemical Oxidants	2006	2008	O_3	8-houir	0.075 ppm	Annual fourth-highest daily maximum 8-hour concentration, averaged over 3 years
Integrated Science Assessment of Ozone and Related Photochemical Oxidants	2013	2015	O_3	8-hour	0.070 ppm	Annual fourth-highest daily maximum 8-hour concentration, averaged over 3 years
Air Quality Criteria for Particulate Matter	1996	1997	$PM_{2.5}$	24-hour	65 $\mu g/m^3$	98th percentile, averaged over 3 years
				Annual	15.0 $\mu g/m^3$	Annual arithmetic mean, averaged over 3 years
			PM_{10}	24-hour	150 $\mu g/m^3$	99th percentile, averaged over 3 years
				Annual	50 $\mu g/m^3$	Annual arithmetic mean, averaged over 3 years
Air Quality Criteria for Particulate Matter	2004	2006	$PM_{2.5}$	24-hour	35 $\mu g/m^3$	98th percentile, averaged over 3 years
				Annual	15.0 $\mu g/m^3$	Annual arithmetic mean, averaged over 3 years
			PM_{10}	24-hour	150 $\mu g/m^3$	Not to be exceeded more than once per year on average over a 3-year period
Integrated Science Assessment for Particulate Matter	2009	2012	$PM_{2.5}$	24-hour	35 $\mu g/m^3$	98th percentile, averaged over 3 years
				Annual	12.0 $\mu g/m^3$	Annual mean, averaged over 3 years
			PM_{10}	24-hour	150 $\mu g/m^3$	Not to be exceeded more than once per year on average over 3 years

[a]In December 2006 EPA announced a revised process for reviewing and setting NAAQS. The changes included the development of the ISA. Previously, the document reporting on EPA's periodic reevaluation of newly available scientific information was referred to as the criteria document (EPA 2016c).
[b]The primary standard is set for protection of public health.
[c]The form defines the air-quality statistic that is to be compared to the level of the standard in determining whether an area attains the NAAQS.

Temporality: CHIE studies have enabled more specific assessment of the timing of responses to short-term exposures to criteria pollutants.

Biologic gradient: Some CHIE studies involving short-term exposures to specific criteria pollutants, particularly those involving ozone (O_3) exposures, have contributed to clarification of exposure–response relationships. In addition, CHIE studies allow for the study of specific gaseous or particle pollutant exposure concentrations and durations.

Plausibility, Experimental Findings, Consistency and Coherence: CHIE studies provide evidence to assess plausibility by assessment of multiple biomarker (see below) and physiologic responses to specific exposures, enabling evaluation of potential mechanisms of action of specific criteria pollutants. CHIE study findings might be used to generate new hypotheses or contribute to the strength of evidence regarding biomarker or physiologic responses to pollutants, when the results are consistent across CHIE studies or when they illustrate coherence with results of toxicologic animal studies or observational epidemiologic studies or panel studies.

However humans are not as identical as inbred mice, leading to differences in interpretation of the meaning of consistency and coherence. Particularly with PM CHIE studies, when lack of consistency or coherence/reproducibility occurs, this may be due to factors other than chance or small number of subjects. These factors can include: (1) variability in the composition of the PM; (2) variability in subject susceptibility. With ozone CHIE studies (see below), internal variability in response was an important piece of information about inter-subject susceptibility to the exposure, and that variability in response was reproducible.

New biomarker or physiologic end points related to cognitive function or other noncardiopulmonary outcomes contribute to evidence for plausibility of epidemiologic associations of criteria pollutants or mixtures with other outcomes that have been less well understood or studied.

SENSITIVE GROUPS

Section 109 of the Clean Air Act indicates that the primary NAAQS should allow for an adequate margin of safety to protect public health. The legislative history of Section 109 indicates sensitive subpopulations (or subgroups) are intended to be a specific focus of efforts to provide such protection.[2] Broadly speaking, sensitive subpopulations comprise individuals who show stronger biologic responses to increased exposure in terms of concentrations and durations, beginning at lower exposure, relative to the general population (that is, sensitive subpopulations exhibit a shifted exposure–response curve). The sensitivity can be attributable to intrinsic factors (such as asthma) or extrinsic factors (such as tobacco smoking). Therefore, CHIE studies can provide information regarding biologic gradients for sensitive subpopulations.

The committee considers sensitive subpopulations to be an important segment of the general population for several reasons. They are a specific focus of the NAAQS requirements in the Clean Air Act. Developing a scientific understanding of the burden of air pollution on them, without causing harm, is a task that requires continuing synthesis of information, as CHIE study protocols are developed and as research plans are formulated. Because sensitive individuals are likely to be biologically vulnerable, they require special attention from Institutional Review Boards (IRBs) that are asked to approve CHIE study plans.

While CHIE studies can inform NAAQS decision making by contributing to the identification of sensitive subpopulations and assessing sensitivity to exposure, the committee has observed that CHIE studies have not included participants with high baseline risks of serious adverse events (see Chapter 4) and finds that it is not warranted to do so in the future (see Chapter 5). Thus many CHIE studies have

[2]Sometimes sensitive individuals are referred to as susceptible or at-risk individuals.

been limited to involving healthy (and often young) adult subjects, whose biologic responses to controlled exposures would likely differ from those of individuals with established disease. Some CHIE studies, which have been conducted after completion of the PM ISA in 2009, have included subjects with metabolic syndrome or mild asthma, and some whose ages are greater than 65 years old (see Chapter 4). However, even though these studies potentially involved somewhat more sensitive individuals, they were designed to exclude individuals who are most likely to have adverse effects (see Chapter 2). While considering this issue, the committee adhered to the principle that, in CHIE studies, the risk of studying people at high baseline risk of an adverse event outweighs the potential benefit of increased scientific understanding accrued to society. Chapter 5 presents recommendations for improving the definition of inclusion and exclusion criteria for selecting study subjects that need to be considered by EPA and the IRB of record.

CHIE STUDIES IN THE CONTEXT OF TOXICOLOGIC AND EPIDEMIOLOGIC STUDIES, AND THE LARGER RESEARCH AGENDA

The primary value of CHIE studies of air-pollutant exposures is that they generate data on responses to short-term criteria-pollutant or pollutant-mixture exposures for well-defined pollutant concentrations and for specific time periods to inform NAAQS with shorter averaging times (such as 8 or 24 hours) (see Table 3-1). Important secondary values include gaining a better understanding of (1) temporal patterns of short-term responses and recoveries, (2) compartments or specific locations in the human body and kinds of cells affected by air-pollutant exposures, and (3) initial and secondary biologic responses as measured by functional physiologic outcomes and biomarkers.

As shown in Figure 3-3, CHIE studies (referred to in the figure as "human challenge studies") and controlled animal inhalation studies (referred to as clinically relevant animal models) provide information to help in the interpretation of the exposure–response relationships generated by panel studies and larger-scale epidemiologic studies of diverse human populations. CHIE studies can provide unique information that cannot be obtained from animal inhalation studies or from epidemiologic or panel studies of people engaged in their normal daily activities in the real world. EPA considers all three sources of complementary exposure–response information in the challenging task of reviewing, and possibly revising, NAAQS. The role that CHIE studies play in supplementing toxicologic studies and observational epidemiologic or panel studies is discussed extensively in EPA's ISAs. For example, see EPA (2009, 2013).

USE OF BIOMARKERS IN CHIE STUDIES

The National Research Council report *Human Biomonitoring for Environmental Chemicals* characterized biomarkers as biologic indicators that generally include biochemical, molecular, genetic, immunologic, or physiologic signals of events in biologic systems (NRC, 2006). There are three broad categories of biomarkers: exposure, response, and susceptibility. As indicated in that report, WHO (2001) defined those categories with respect to environmental chemicals as follows:

> *Biomarker of exposure*. The chemical or its metabolite or the product of an interaction between a chemical and some target molecule or cell that is measured in a compartment in an organism.
>
> *Biomarker of effect*. A measurable biochemical, physiologic, behavioral, or other alteration in an organism that, depending on the magnitude, can be recognized as associated with an established or possible health impairment or disease.
>
> *Biomarker of susceptibility*. An indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical substance.

Figure 3-2 shows various classes of biomarkers that can be considered across the steps of an exposure–response sequence. For example, biomarkers of exposure can be used to estimate the concentration of a toxicant in the breathing zone of an individual, and to distinguish it from the dose of the toxicant that is delivered to the airway surface or to a target organ and even to the target receptor for a specific mechanism of action.

In choosing the biomarkers as study end points that focus on perturbations of concern for short-term effects, an important consideration is the short-term effects that might be indicative of the initiation and progression of chronic effects (NRC 2007). The use of a broad array of biomarkers allows scientists in other disciplines, such as exposure scientists, epidemiologists, and toxicologists, to anchor their studies with specific biomarkers.

Biomarkers of short-term responses detected in CHIE studies might be useful in other complementary studies, such as panel studies of human cohorts to assess variations in biologic responses in specific subpopulations, including potentially susceptible subpopulations, to relatively short-term exposures to ambient pollutant mixtures. Biomarkers detected in CHIE studies also might be useful for chronic inhalation exposure studies involving animals, in that seeing similar biomarkers in animals and humans could provide some validation of the animal studies for use in characterizing human risk associated with exposure to air pollutants. However, some biomarkers that are unique to long-term effects might not be identified through CHIE studies. Biomarkers of short-term responses in CHIE studies might also be useful in large-population epidemiologic studies to identify subpopulations at relatively high risk of developing clinically relevant pollutant-induced chronic disease that could benefit from preventive medical intervention.

COMPARING CHIE STUDY EXPOSURES WITH AMBIENT POLLUTANT EXPOSURES

The relevance of the results of controlled inhalation exposures to the potential effects of exposure to similar criteria pollutants in ambient air can vary depending on

- Whether the criteria pollutant represents a variable mixture or has more than one molecular form,
- The complexity and variety of effects of concern, and
- The presence of hazardous air pollutants that co-occur with the criteria pollutant in ambient air.

For carbon monoxide (CO), a criteria pollutant that is in a singular molecular form and whose metabolic products, i.e., carboxyhemoglobin and carboxymyoglobin, are risk factors for a specific adverse health effect, the similarity between controlled exposures and ambient exposures is expected to be very high.

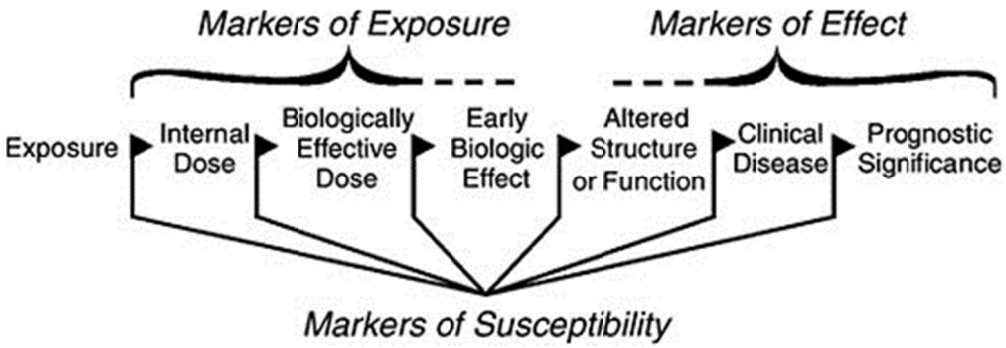

FIGURE 3-2 Simplified flow chart of classes of biomarkers. Source: Adapted from NRC (1987).

Other current criteria pollutants represent mixtures of ambient air pollutants, including multiple known toxicants in gaseous and particulate forms, such as peroxides as well as O_3 for photochemical oxidants, NO and HNO_3 as well as NO_2 for nitrogen oxides, SO_3 and H_2SO_4 for sulfur oxides, and toxic trace metals and complex polycyclic aromatic hydrocarbons for PM.

The nature of the measurable health-related responses observed in O_3 CHIE studies is most similar to those reported in time-series studies of ambient air O_3 exposures. It has been established that the magnitude of the pulmonary function responses, per ppb of O_3, is greater for ambient air exposures than for exposures in CHIE studies (Spektor et al., 1988). A common interpretation of that finding is that copollutants are also playing a causal role.

$PM_{2.5}$ is the best example of a criteria pollutant where of the results of controlled laboratory exposures and ambient air exposures tend to be most variable. The epidemiologic evidence demonstrates that there are variable exposure–response relationships, for both acute and chronic responses, between and within cities. Many studies have found that relative toxicity of PM corresponds to the differences in the chemical composition of the PM (see, for example, Thurston et al., 2013, 2016a), or to the sources that the chemical composition represents. However, much is still to be understood about how PM composition influences toxicity. Furthermore, in ambient air, there is always simultaneous exposure of $PM_{2.5}$, photochemical oxidants, sulfur oxides, and nitrogen oxides in various proportions, and the health effects associated with $PM_{2.5}$ exposures can be influenced by its copollutants (Lippmann et al., 2013). For CHIE studies of $PM_{2.5}$ mass concentrations from diesel-engine exhaust (DE), the applicability of the results for to the potential effects of ambient air exposures where there are few sources of DE is more tenuous, because $PM_{2.5}$ from DE is much richer in organic carbon (OC) and much poorer in transition metals (such as iron and nickel) than is ambient air $PM_{2.5}$.

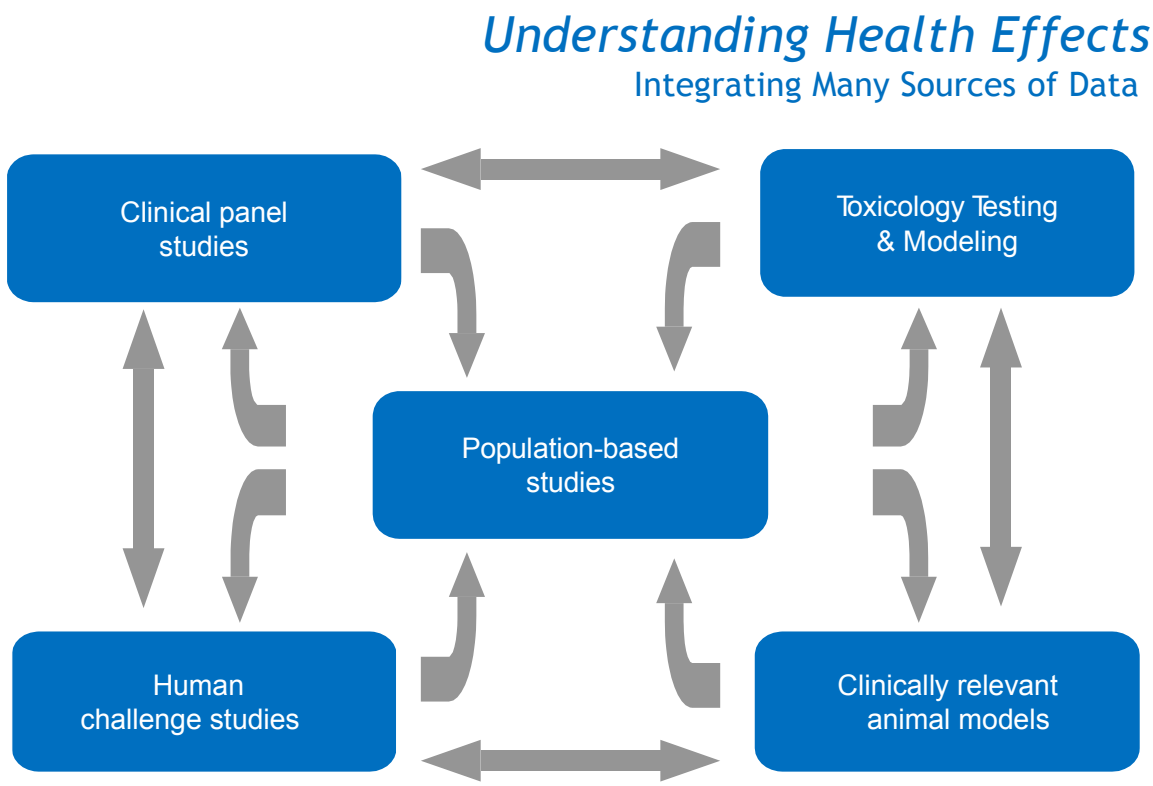

FIGURE 3-3 Pathways for integrating health-effects data. Source: Adapted from EPA presentation to committee June 1, 2015.

Controlled Human Inhalation-Exposure Studies at EPA

CONSIDERATIONS OF CHIE STUDY VALUE FOR EPA DECISION MAKING

The value of CHIE studies for informing the reviews of NAAQS for O_3 and $PM_{2.5}$, and for understanding biologic responses to airborne PM from specific emission sources or of specific compositions, is discussed below.

CHIE studies have been carried out to obtain a detailed understanding of the impact of O_3 from two perspectives. One perspective focuses on the considerations of specificity, temporality, and biologic plausibility for providing justification for establishing NAAQS for O_3 as an indicator pollutant that is associated with human harm. The second perspective focuses on the consideration of a biologic gradient and connects to the regulator's task of establishing an averaging time, level (mean concentration over the specified averaging time), and statistical form for a NAAQS, as discussed below.

For airborne PM, scientific investigations and understanding of health impacts occurred through a somewhat different historical route. The epidemiologic evidence of mass-based PM-related health effects (particularly cardiac health effects related to inhalation of PM and entry into the lung) was initially greeted with skepticism and hence CHIE studies have been used to provide evidence for specificity, temporality, and biologic plausibility. There has been less emphasis on consideration of biologic gradient, and investigation of gradient is complicated by the variable complex chemical composition, particle-size distribution, and/or source of a given level of PM.

For O_3 and PM, the following discussion provides (1) background on the value of CHIE studies for NAAQS decision making, (2) a summary of CHIE study contributions to the evidence provided in the ISAs (and in select cases, assessments by other expert panel reviews), and (3) a summary of CHIE study contributions to understanding biologic gradients and informing decisions concerning the four basic elements of the NAAQS. The discussion of PM CHIE studies also includes a consideration of the influence of particle size range and chemical composition for NAAQS decision making.

CHIE OZONE STUDIES

Background on the Value of CHIE Studies for the Ozone NAAQS

CHIE study findings have been valuable in informing O_3 NAAQS decision making. The strongest evidence for O_3-associated health effects is for respiratory effects following short-term exposures. The ISA (EPA, 2013; Table 1-1) concludes that for short-term O_3 exposures, evidence supports a causal relationship with respiratory effects and is highly suggestive of a direct or indirect contribution to cardiovascular effects and premature mortality. CHIE studies demonstrated a wide range of respiratory effects, including lung-function decrements and increases in respiratory symptoms, lung inflammation, and airway hyperresponsiveness.

Specificity, Temporality, and Plausibility Considerations

As indicated in the O_3 ISA, most CHIE studies investigating the effects of O_3 exposure used a randomized, controlled, crossover design in which subjects were exposed, without knowledge of the experimental treatment and in random order to clean filtered air (FA) as the control and, depending on the study, to one or several O_3 concentrations, frequencies, and durations. The control exposure provides a direct estimate of the effects of the experimental conditions on the biomarker or physiologic outcomes of interest. Comparison of biologic responses to the FA exposure to those following an O_3 exposure allows for estimation of the O_3 effects, while controlling for independent effects of the experimental procedures. As individuals may experience small changes in various health end points from exercise, diurnal variation, or other influences, in addition to those of O_3 during the course of an exposure, the term "O_3-induced" is used to designate effects that have been corrected or adjusted for such extraneous responses as measured during FA exposures (EPA 2013).

The initial end points used for the historic CHIE studies were spirometric (lung function) indices obtained via pulmonary function testing. Those indices characterized reproducible physiologic characteristics of individuals, with normal values being dependent on an individual's age, sex, and height (as an index of lung volume). An extensive body of literature has documented their reproducibility in individual subjects, and they are used not only for human health-effects research related to air pollution exposure, but are used longitudinally for pharmacologic studies as a primary end point by the Food and Drug Administration. Reduced respiratory function is also an independent risk factor for mortality as determined by epidemiologic studies unrelated to those focused on air pollution (Agarwal et al., 2012; Helzebos et al., 2014; Hozawa et al. 2006; Lee et al. 2011; Menezes et al. 2014; Shaaban et al, 2006; Sin et al. 2005) . This measure additionally has been used to stratify the severity of common respiratory diseases including asthma, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis.

Initial CHIE studies looked at responses to O_3 breathed in by subjects at rest. Subsequent studies evaluated the impact of O_3 inhalation under conditions of moderate exercise and, more recently, under conditions of high ambient temperature. The rationale for evaluating O_3 exposure under conditions of exercise is related to the recognition that individuals working outside or engaged in recreational exercise or athletic competition would be expected to breathe at higher than normal minute ventilation and would therefore have a higher internal dose rate. CHIE studies provide an ideal setting to study how exercise modifies the short-term adverse effects of O_3 exposure. It should be noted that comparative studies between humans and nonprimates (rodents) showed quite different exposure–response relationships. The differences in breathing pathways and rates, as well as patterns and targets of injury, between rodents and humans resulted in an enhanced appreciation for the value of the CHIE study model.

CHIE studies evaluated the impact of controlled exposure to O_3 at progressively lower concentrations and with multihour exposure durations designed to mimic a typical day's work outside. These studies demonstrated several important findings. The first was an average decline in lung function that became progressively greater with continuing exposure over a 6.6-hour period, with the subjects engaged in intermittent moderate exercise. This finding suggested the need for averaging times of 8 hours duration, whereas the previous NAAQS had a 1-hour averaging time. A second key finding from the CHIE O_3 exposure studies was the demonstration of a high degree of interindividual variability in the magnitude of the short-term spirometric decrements with O_3 exposure. This finding was unexpected in its magnitude, with interindividual variability of 40% or greater within a predicted average FEV_1decrement of 10% for white males aged 18-36 years for 2-hour exposures with intermittent exercise (McDonnell et al., 1997). This result led to the recognition that small average changes in a population masked or obscured variability across the population. This finding stimulated observational population-based epidemiologic investigations of clinical end points, such as respiratory or cardiovascular hospitalizations and exacerbations.

The observation of large interindividual variation in spirometric decrements led to an assessment of whether this was a stable response phenotype. Other studies demonstrated that there was intraindividual stability in the spirometric responses to O_3, even while there was persistent large interindividual variability (Folinsbee et al., 1994; Hazucha et al., 2003; McDonnell, 1996; McDonnell et al., 1985).

These studies spurred an avenue of animal toxicologic studies by Kleeberger (1995) evaluating genetic determinants of O_3 responsiveness, a productive line of research that continues at this time.

Another important contribution of CHIE studies of O_3 was the evaluation of the inflammatory response to controlled exposure to O_3. Key papers evaluating bronchoalveolar lavage (BAL) profiles after O_3 exposure demonstrated perturbations of the alveolar–capillary interface with influx of inflammatory cells, plasma transudation into the alveolar space, and activation of inflammatory cascades, including the perturbation of the coagulation system. The value of the contributions stem from the simplicity of the exposure, unclouded by concomitant exposures to other toxicants in the ambient air mixture, and the experimental design of CHIE studies. Subsequent epidemiologic studies have developed the knowledge base of the implications of these perturbations in sensitive subpopulations (Alexis et al., 2010; Devlin et al., 1991; Kim et al., 2011; Koren et al., 1989; Lay et al., 2007).

Another line of investigation of O_3 exposure examined the relationship between physiologic and inflammatory responses. This provided useful initial information in interpreting time trends in pulmonary

function response to O_3. The CHIE studies demonstrated a diminution of the physiologic response with successive daily exposures ("O_3 adaptation"). It was unknown whether this was a beneficial response or a manifestation of harm, and CHIE studies allowed an examination of that question. The finding was that the inflammatory response persisted despite a lessening of the lung function decrement. This, in turn, led to the appreciation of dimensions of response to pollutants and cautioned against an overly simplistic interpretation of any single study (Folinsbee et al., 1980).

A subsequent area of investigation in CHIE studies was an exploration of the impact of O_3 exposure on the inflammatory response of individuals with preexisting inflammatory airway disease, such as asthma and COPD. Asthma is a syndrome, and great progress is being made in the elucidation of asthma phenotypes, including the role of atopy, obesity, hormonal status (that is, postmenopausal), and viral infection on the inflammatory and physiologic characteristics of asthma. It is important to understand the impact of O_3 on these distinct phenotypes, as their pathogenesis is deciphered (Alexis et al., 2000; Hernandez et al., 2010; Peden et al., 1995, 1997).

Similarly, COPD is increasingly understood to be a syndrome with subphenotypes (Kleeberger and Peden, 2005; Speizer and Ware, 2015). One of the potential values of CHIE O_3 studies is to understand whether perturbations in the inflammatory profile are similar for these different phenotypes, or distinct. The Clean Air Act has, as a founding principle, the intent to protect sensitive subpopulations with a reasonable margin of safety. CHIE studies with O_3 provide an ongoing means to understand and refine the notion of sensitivity. An example of this is more recent findings of association of O_3 effects and ambient temperature (Kahle et al., 2015).

Because of epidemiologic observational evidence of strong cardiovascular effects associated with PM rather than with O_3, the past decade of CHIE research has focused more on assessment of cardiophysiologic effects of $PM_{2.5}$, rather than O_3. Relatively recent time-series epidemiology studies have reported statistically significant associations not only between PM exposure and daily mortality and/or morbidity due to pulmonary and/or cardiovascular causes, but also between daily ambient O_3 concentrations and those outcomes (Basu, 2009; Basu and Malig, 2011; Bell et al., 2004; Ito et al., 2005; Katsouyanni et al., 2009; Rosenthal et al., 2013; Stafoggia et al., 2010; Zanobetti and Schwartz, 2008). Those observations have led EPA investigators to turn to CHIE studies to assess whether there is biologic evidence to support the associations of short-term O_3 exposures with clinical outcomes that have been observed through epidemiologic studies. A CHIE study involving sequential 2-hour exposures to clean air and O_3, at 22°C and again at 32.5°C, showed an interaction between high temperature and O_3 that may activate the fibrinolytic pathway and help to explain the adverse effect of O_3 on cardiac mortality and morbidity (Kahle et al., 2015). A CHIE study of young, healthy adults found that O_3 can cause an increase in biomarkers of vascular inflammation and changes in markers of fibrinolysis and markers that affect autonomic control of heart rate and repolarization (Devlin et al. 2012).

CHIE studies of subjects at rest have contributed to NAAQS decision making by confirming the reproducibility of physiologic changes associated with O_3 exposures. CHIEs studies have contributed to the understanding of O_3 effects on lung function: that O_3 inhibits the ability to inspire to total lung capacity (Hazucha et al. 1989) thereby reducing FEV_1 and FVC. That information helps greatly in understanding the effects on lung function estimated in observational studies. CHIE studies have also demonstrated physiologic effects of O_3 exposures under outdoor working conditions, such as elevated ambient temperatures.

Biologic Gradient Considerations Ozone CHIE Study Contributions to the Four Basic Elements of the NAAQS

This section focuses on the use of O_3 CHIE studies to establish biologic gradients for O_3-associated health effects in order to inform decisions about the primary photochemical oxidant standard. The most recent ISA for O_3 was completed in 2013 (EPA, 2013). As indicated in Table 3-1, the components of the current primary NAAQS, set in 2015, include O_3 as an indicator, an 8-hour averaging time, a concentration of 70 ppb O_3, and a form defined as the annual fourth-highest daily maximum averaged over 3 years. Below we discuss how CHIE studies of O_3 contributed to each of these components of the NAAQS.

Indicator: The ISA for O_3 noted that O_3 and NO_2 are the only photochemical oxidants that are routinely monitored and for which a comprehensive ambient air concentration database exists. The findings from CHIE studies, discussed above, provide a sound justification for the selection of O_3 as an indicator pollutant. As air pollution oxidant chemistry becomes better understood, opportunities will arise for CHIE studies to address other photochemical oxidants in ambient air, especially peroxides.

Averaging time: CHIE studies provide a basis for evaluating the appropriateness of a primary NAAQS with an 8-hour averaging time, instead of using the shorter exposure duration (1 hour) that was used in earlier O_3 NAAQS. The change to an 8-hour averaging time was based on earlier CHIE studies that investigated 6.6- and 8-hour exposures in healthy adults and reported respiratory effects at lower O_3 exposure concentrations for 1- and 2-hour exposures with moderate levels of exertion (for example, see McDonnell et al., 1991). O_3 causes an inflammatory response in the lungs after a single 1-hour exposure (with exercise) to O_3 at a concentration of 300 ppb and that the increased concentrations of some inflammatory cells and mediators persisted for at least 18 hours.

It should be noted that the 6.6-hour exposure study has reported respiratory effects below the lowest effective O_3 dose as determined during a 1-hour exposure study in young healthy adults. That suggested that ambient O_3 had cumulative daily effects and/or that a longer averaging time than 1 hour is necessary to protect populations from O_3's accumulated effects (McDonnell et al., 1991), thus motivating a longer averaging time for NAAQS for O_3 (see below).

Some CHIE studies have been designed to evaluate specific exposure circumstances of interest to regulators. For example, outdoor workers engaged in heavy physical labor were identified as a potentially sensitive subpopulation, and the 6.6-hour experimental protocol was intended to simulate this condition (Folinsbee et al., 1988). The subsequent 8-hour average time for O_3 is similar to the exposure periods investigated in this 6.6-hour exposure study.

Ambient O_3 concentrations during any 24-hour period vary, with peaks generally occurring in the late morning and/or early afternoon. The timing of the 1-hour maximum concentration could be affected by unusual, sudden increases in the background O_3 concentration. The choice of an 8-hour average represents a compromise that takes into account evidence from CHIE studies as well as the necessities of risk management through regulatory implementation.

Level: The current concentration limit for the primary O_3 NAAQS was reduced from 0.075 to 0.070 ppm in 2015, based on complementary information from CHIE, epidemiologic, and panel studies. CHIE studies provided essential information on exposure–response relationships for various O_3 concentrations for durations up to 8 hours. Available evidence from CHIE 6.6-hour studies show that detectable effects of O_3 at constant exposure during the study time on group mean FEV_1 (forced expiratory volume in 1 second) were observed at exposure concentrations as low as 60 ppb, but effects were not observed at 40 ppb, in young healthy adults exposed for 6.6 hours while engaged in moderate exercise (EPA, 2013).

Form: The "form" of a NAAQS defines the air-quality statistic (such as the annual fourth-highest daily maximum 8-hour concentration, averaged over 3 years; see Table 3-1) that is to be compared to the level of the standard in determining whether an area attains the NAAQS. EPA indicates the main consideration in selecting a form for current standards is the adequacy of the public health protection provided by the combination of the four elements of the standard (EPA, 2014). The selection of the form of a standard is mainly based on the daily distribution of ambient O_3 and risk management target rather than the dose–response relationship obtained from CHIE studies. Also, as mentioned previously, those studies have been complicated by the geographically and temporally variable composition of ambient PM.

Controlled Human Inhalation-Exposure Studies at EPA

Impacts of the Available Results of CHIE Ozone Studies on the Ozone NAAQS

The ISA provides a synthesis and evaluation of the policy-relevant studies as the scientific foundation for the periodic review of the NAAQS required by the Clean Air Act. The primary NAAQS for O_3 and related photochemical oxidants is designed to protect against respiratory health effects incurred after short-term exposure to tropospheric (ambient) O_3 and related photochemical oxidants.

O_3 CHIE studies have been of critical importance for informing NAAQS decision making by providing

- A basis for EPA's decision to move from a 1-hour to an 8-hour averaging time for O_3 concentration. For example, there was the finding of concentration-dependent increases in BAL neutrophils and the inflammatory mediator IL-6 for 6.6-hour exposures to O_3 at moderate concentrations (0.080 and 0.10 ppm; Devlin et al., 1991);
- An understanding of the role of risk factors in human physiologic and biologic responses to oxidant pollutant exposures:
  - Some individuals in CHIE studies showed no change in lung function while others showed up to a 30% decrease in lung function after a 6-hour O_3 exposure. The phenotype of responding to O_3 exposure with a decrease in lung function was shown to be reproducible.
  - Some individuals in CHIE O_3 studies responded with increases in markers of lung inflammation. These were not always the same individuals as those who responded to O_3 with a decrease in lung function;
- An understanding of O_3 adaptation. Lung function responses to O_3 decreased after repeated daily O_3 exposures, but the inflammatory response was sustained over repeated exposures (Devlin et al., 1997);
- Identification of decreased lung function, increased airway inflammation, and increased respiratory symptoms in healthy adult subjects after controlled exposure to O_3 concentrations less than 75 ppb (EPA, 2014);
- Evidence to support the plausibility of elevated ambient O_3 exposures causing increased asthma events observed in sensitive "at-risk" subpopulations;
- Evidence of O_3-related health response presented in the 2006 O_3 air-quality criteria document (EPA, 2006), providing support for a causal relationship between acute ambient O_3 exposures and increased respiratory morbidity outcomes, and the 2013 ISA's conclusion that it is a causal relationship, providing support for lowering the O_3 NAAQS;
- Biologic and physiologic evidence for O_3 effects in human health that generated hypotheses for animal studies that looked for risk factors in complementary investigations; and
- An iterative process in which the results of CHIE studies inform the efforts of interdisciplinary teams working to elucidate biologic mechanisms, and those teams identifying new questions to be addressed by CHIE studies.

CHIE PM STUDIES

Background on the Value of CHIE Studies for the PM NAAQS

CHIE study findings have been used to inform decisions about setting the NAAQS for $PM_{2.5}$ and PM_{10}. As CHIE studies involve short-term exposures and biologic outcomes, they have been specifically relevant to ISA reviews of short-term effects of ambient PM exposure and to the setting of NAAQS related to those effects. In 2009 the most recently completed ISA document (EPA, 2009) cited contributions of CHIE studies in elucidating cardiovascular, respiratory, and other effects (see Chapter 6 and Annex C of the ISA). In 2012, EPA issued a "Provisional Assessment of Recent Studies on Health Effects of Particulate Matter Exposure" (EPA, 2012). An updated version of the PM ISA is in development.

NAAQS have been established for both PM_{10} and $PM_{2.5}$ (see Table 3-1). However, as stated in the 2009 ISA and subsequent American Thoracic Society reviews, the specific contribution of the thoracic coarse fraction of PM_{10} (particles with diameters greater than 2.5 μm and less than or equal to 10 μm [$PM_{10-2.5}$]) to health outcomes and intermediate physiologic or biomarker outcomes is less well understood than the contribution of $PM_{2.5}$, and the effects of the coarse fraction of PM_{10} is an active area of investigation.

The 2009 ISA showed that many CHIE studies provided evidence of biologic plausibility of outcomes observed in time-series epidemiologic studies of short-term responses conducted in the United States and elsewhere by demonstrating perturbations in pathways that are relevant to the development of clinical effects. CHIE study results also showed congruence with outcomes demonstrated in animal toxicity studies. Integrating the complementary data from CHIE studies with observational epidemiologic studies and animal toxicity studies, the 2009 ISA found that the strongest evidence for PM-associated health effects was for associations of short-term exposures to $PM_{2.5}$ with overall mortality, cardiovascular mortality, and nonfatal events. The ISA also found there was some evidence for respiratory effects associated with short-term $PM_{2.5}$ exposures. Based on all the evidence, the 2009 ISA concluded there are causal relationships between short-term $PM_{2.5}$ exposure and cardiovascular effects and mortality, and that the relationship is "likely to be causal" for short-term $PM_{2.5}$ exposure and respiratory effects (EPA, 2009, Table 2-1).

CHIE studies of O_3 involve exposure to a discrete chemical entity. In contrast, CHIE PM mass studies involve exposure to a complex mixture that varies both temporally and spatially in the real world. In its review of the PM NAAQS that was completed in 2012, EPA indicated: "We recognize that important uncertainties remain in this review related to understanding the temporal and spatial variability in $PM_{2.5}$ concentrations, including $PM_{2.5}$ components, and associated health impacts across different geographic areas and seasons" (EPA, 2011, pp. 2-25).

However, current $PM_{2.5}$ and PM_{10} concentration regulations are based only on particle mass, and such regulations, based on observed reductions in particle mass concentration, have been associated with quantitative improvements in mortality and morbidity in settings with PM of varying chemical and biologic components (Correia et al., 2013; Dockery and Ware, 2015; Gauderman et al. 2015; Hao et al., 2017; Laden et al. 2006; Lepeule et al. 2012; Pope et al., 2009, 2013). Given the available information, EPA had decided to maintain the mass-based PM standards during the PM NAAQS review completed in 2012. Quoting directly from the 2009 ISA:

> "Overall, the results … indicate that many constituents of PM can be linked with differing health effects and the evidence is not yet sufficient to allow differentiation of those constituents or sources that are more closely related to specific health outcomes. These findings are consistent with the conclusions of the 2004 PM AQCD (EPA[,] 2004), that a number of source types, including motor vehicle emissions, coal combustion, oil burning, and vegetative burning, are associated with health effects. Although the crustal factor of fine particles was not associated with mortality in the 2004 PM AQCD, recent studies have suggested that PM (both $PM_{2.5}$ and $PM_{10-2.5}$) from crustal, soil or road dust sources or PM tracers linked to these sources are associated with cardiovascular effects. In addition, secondary [sulfate] $PM_{2.5}$ has been associated with both cardiovascular and respiratory effects."

That conclusion was reaffirmed by EPA's "Provisional Assessment of Recent Studies on Health Effects of Particulate Matter Exposure" (EPA, 2012).

The impact of PM chemical composition variability on human toxicity is an important issue that is relevant to EPA's regulatory task (for example, see Bell et al., 2009; Boehm et al., 2015; Cox and Popken, 2015; Dominici et al., 2015; Enstrom, 2005; Greven et al., 2011; Kioumourtzoglou et al., 2015; Young and Xia 2013). If a future ISA concludes that the overall body of research is sufficient to identify regional differences in PM toxicity, then future regulatory approaches that differ by region might be warranted rather than a single, nationwide PM mass-based standard.

Controlled Human Inhalation-Exposure Studies at EPA

In an effort to inform future reviews of the PM NAAQS, it would be impractical to use the CHIE study approach to examine the impact of the full range of PM compositions and dose ranges on biologic perturbations associated with ambient PM exposure. An important research strategic planning task is deciding how to address the range of possible PM compositions for future CHIE studies to increase the understanding of the relative importance of PM components on human toxicity for the purposes of regulation (see Chapter 5).

Specificity, Temporality, and Plausibility Considerations

Because the current PM ISA was completed in 2009 and the next iteration of the document is in preparation, the committee examined additional more-recent publications, including reviews of the state of the art on cardiovascular effects of ambient air pollution, for example, Sun et al. (2010), Crouse et al. (2012), EPA (2012), Hoek et al. (2013), Gold and Mittleman (2013), and Lippmann (2014).

Specificity and temporality: As with the O_3 studies cited earlier in the chapter, the majority of CHIE studies investigating the effects of PM exposure involved a randomized, controlled crossover design with random assignment of exposure sequence to clean FA as the control and to one of several possible PM exposures. Comparison of response following an FA exposure to those following a PM exposure allows for estimation of the PM effects on an outcome measure while controlling for independent effects of the experimental procedure, and corrected for small changes due to exercise or other influences. This study design is fundamental to the value of the CHIE study in providing specificity, that is, specifically connecting the exposure of interest with the biologic outcomes, while removing confounding factors. It also provides information on temporality, unequivocally connecting the pollutant exposure to biologic outcomes, excluding the possible influence of diurnal variation through the crossover design with the FA control.

PM CHIE studies have examined a variety of exposures, depending on the location of the study facility, and whether the PM generation method involves the concentration of PM from the ambient air, by resuspension or instillation of source particles that are brought to the study site from different locations, or by onsite generation of PM from a specific source, such as diluted diesel-engine exhaust or wood smoke. The selection of the PM source depends on the goals of the specific protocol. Results of high relevance to the PM NAAQS come from CHIE studies with inertially concentrated airborne particles (CAPs) into a small fraction of the original ambient air volume. CAPs contain elemental carbon (EC), which is a ubiquitous single component of airborne PM, which has frequently been associated with adverse health effects in epidemiologic studies. Another particle fraction is OC, which usually adds more mass to $PM_{2.5}$ than does EC.

All of the EPA CHIE studies that were cited in the 2009 PM ISA involved exposures to CAPs, as did other studies cited in the ISA that were conducted by other investigators in California and Canada. The cited studies, which involved laboratory-generated EC rather than CAPs, were also performed in U.S. laboratories, with most of them performed by investigators at the University of Rochester. In contrast, nearly all of the cited studies involving controlled human exposures to diluted motor-vehicle engine exhaust were conducted in European countries, which have had different regulations affecting motor vehicle engine exhaust.

Plausibility: The 2009 ISA cited CHIE studies extensively regarding associations between short-term PM exposure and biologic end points. For cardiovascular and systemic effects, CHIE studies were cited in support of the plausibility of these biologic end points:

- Heart rate variability;
- Vasomotor function;
- Systemic inflammation;
- Hemostasis, thrombosis, and coagulation factors; and
- Systemic and cardiovascular oxidative stress.

For respiratory effects, CHIE studies were cited in support of the plausibility of these biologic end points:

- Respiratory symptoms and medication use,
- Pulmonary function,
- Pulmonary inflammation,
- Pulmonary oxidative responses,
- Pulmonary injury, and
- Allergic responses.

The American Heart Association Statistical Update (Mozaffarian et al. 2016) provides recent information on cardiovascular health; a range of major clinical disease conditions (including stroke, congenital heart disease, rhythm disorders, subclinical atherosclerosis, coronary heart disease, heart failure, valvular disease, and peripheral arterial disease).

By evaluating cardiophysiologic and biomarker outcomes associated with CAPs exposures, CHIE studies have provided human studies data on the mechanistic plausibility of prior epidemiologic observations suggesting that short-term exposure to elevated concentrations of ambient PM adversely affected cardiac health. Thus CHIEs contributed to understanding how PM inhalation exposure could enter the lung and affect the heart. Figure 3-4 provides a paradigm to frame hypothesis testing, but it does not by any means include all the layers of current understanding of the pathogenesis of various CVD outcomes. While useful, this paradigm is likely to be modified by continuously evolving understanding of CVD etiology.

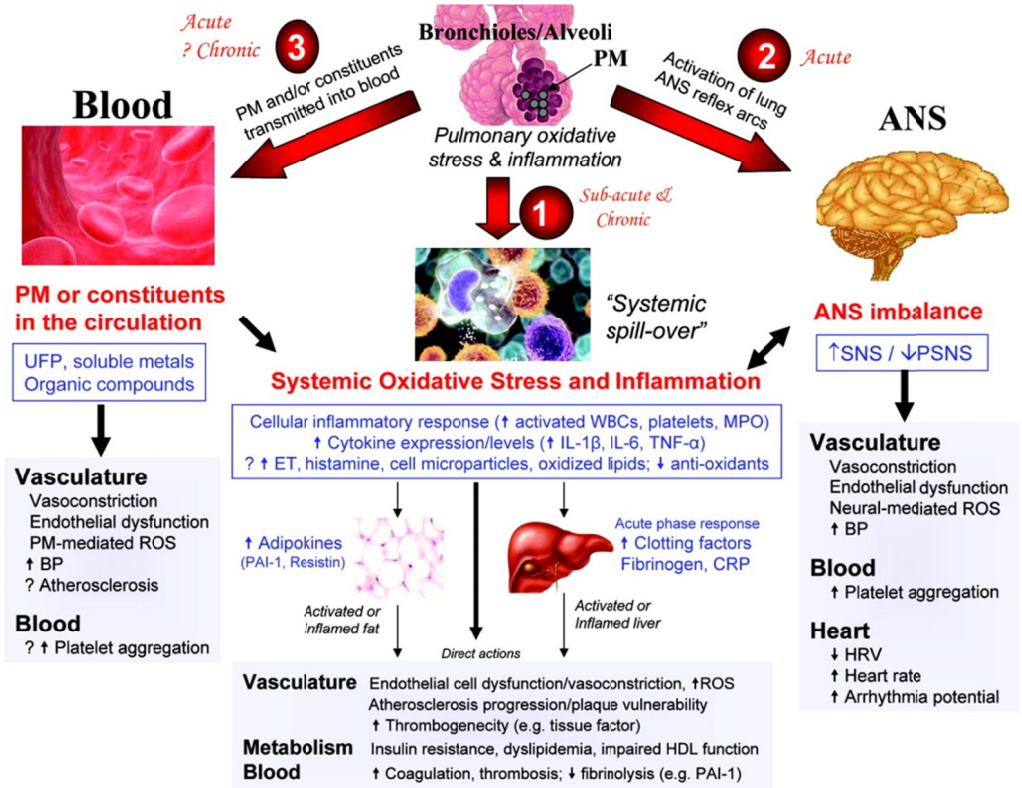

FIGURE 3-4 Systemic oxidative stress and inflammation. Source: Brook et al. (2010). Reprinted with permission; copyright 2010, American Heart Association.

Controlled Human Inhalation-Exposure Studies at EPA

Specific Cardiovascular Responses

Heart rate variability (HRV): HRV is a measure or marker for cardiovascular autonomic function. Up to a point, more HRV is needed to enable flexibility in response to cardiophysiologic challenges. In longitudinal observational cardiovascular epidemiology studies, reduced HRV (assumed to be chronic) has been a predictor of increased cardiovascular risk. However, it is uncertain in those studies whether HRV is a marker for general ill health or whether it actually influences long- term outcomes directly. CHIE studies have evaluated the acute influence of PM on short-term reversible changes in this physiologic outcome, as part of testing of the plausibility that PM that enters the lung could lead to small reversible perturbations in cardiovascular autonomic function.

There was limited evidence identified in the 2009 ISA to suggest that acute exposure to PM might be associated with HRV changes, suggesting plausibility that particles entering the lung could affect vascular function. With some suggestion of consistency across study designs, many, but not all, animal studies, and human panel studies, as well as CHIE studies, reported altered autonomic function measured by HRV in response to PM. Some studies showed reduced HRV (increased parasympathetic activity relative to sympathetic activity), with other studies showing autonomic responses in the opposite direction. In the CHIE studies PM exposure was most consistently associated with reduced HRV in healthy older adults (Devlin et al., 2003; Gong et al., 2004).

Vasomotor function: In addition to HRV, CHIE studies, as well as observational population-based and panel studies of healthy adults and adults with cardiovascular disease risk factors, have evaluated macrovascular and microvascular subclinical physiologic fine and coarse PM responses with potential relevance to cardiovascular function. Some macrovascular outcomes have included brachial artery diameter, branchial artery flow-mediated dilation (FMD), and blood pressure. The literature was reviewed in the 2009 ISA and in 2010 in an AHA scientific statement (Brook et al. 2010). The AHA statement evaluated the published CHIE studies on PM and physiologic vascular outcomes, including ones in Toronto and Michigan demonstrating associations of concentrated traffic fine particles on brachial artery vasoconstriction or blood pressure in healthy adults (Brook et al. 2002, 2009). While acute subclinical macrovascular responses to PM were not consistently found across all study designs and population, the AHA statement concluded that "even when the few negative studies are considered, the overall evidence supports the concept that ambient PM is capable of impairing vascular function, particularly among higher-risk individuals (for example, those with diabetes [Schneider et al. 2008]) and after traffic-related exposure (Brook et al. 2010, page 2347)."

The 2009 ISA indicated that the cumulative results of several CHIE studies suggest that exposure to diesel exhaust particles (DEPs) is associated with inhibition of endothelium-dependent and endothelium-independent vasodilation (within 2-6 hours), and that the suppression might remain up to 24 hours following exposure (Lund et al., 2009; Mills et al., 2005, 2007; Peretz et al., 2008; Tornqvist et al., 2007). In patients with coronary artery disease, vasodilator function was not affected 6-8 hours after exposure. Shah et al. (2008) suggested that ultrafine particles of EC might produce small changes in systemic vascular function.

Plausibility for systemic inflammatory, prothrombotic, and oxidative stress responses: The ISA and the subsequent American Heart Association (AHA) expert panels reviewed CHIE and other studies on these classes of outcomes.

Systemic inflammation*:* The ISA reported that CHIE studies of exposures to various PM types have provided limited but inconsistent evidence of a PM-induced increase in markers of systemic inflammation (Barregard et al., 2006; Beckett et al., 2005; Blomberg et al., 2005; Bräuner et al., 2008; Carlsten et al., 2007; Frampton et al., 2006; Gong et al., 2004a,b, 2008; Graff et al., 2009; Mills et al., 2005, 2007, 2008; Peretz et al., 2007; Routledge et al., 2006; Samet et al., 2009; Tornqvist et al., 2007).

Hemostasis, thrombosis, and coagulation: The ISA reported that some CHIE studies provided evidence that short-term exposure to $PM_{2.5}$ might have small yet statistically significant effects on hemostatic

markers in healthy subjects or patients with coronary artery disease (Barregard et al., 2006; Graff et al., 2009; Lucking et al., 2008; Mills et al., 2005, 2007; Samet et al., 2009).

Systemic and cardiovascular oxidative stress: The ISA reported that CHIE studies of exposure to $PM_{2.5}$ might increase systemic oxidative and inflammatory responses in human subjects (Barregard et al., 2006; Bräuner et al., 2007; Peretz et al., 2007; Tornqvist et al., 2007).

The ISA reported that a number of CHIE studies suggested that $PM_{2.5}$ might increase systemic oxidative and inflammatory responses in human subjects. However studies were relatively few with limited consistency in results, as well as in outcomes measured. Subsequently, more published panel studies and CHIE studies have suggested associations of $PM_{2.5}$ with oxidative stress or inflammatory responses, including some but not all studies reviewed in a 2010 scientific statement from the American Heart Association (Brook et al. 2010). Regarding oxidative stress, statement concluded: "Although not entirely consistent, the available studies demonstrate that acute exposure to PM, perhaps even at ambient levels, may be capable of inducing acute systemic oxidative stress in human subjects under certain circumstances. The assays used to assess the footprint of systemic "oxidative stress" or damage may also play a significant role in the results" (Brook et al. 2010, page 2360).

Plausibility for respiratory responses: Plausibility for respiratory responses was also reviewed by the 2009 ISA and subsequent AHA expert panels.

Symptoms: The ISA reported that CHIE studies found no association between short-term $PM_{2.5}$ exposure and respiratory symptoms.

Pulmonary function: The majority of CHIE studies cited in the ISA did not provide evidence of $PM_{2.5}$-induced changes in pulmonary function; however, some investigators observed slight decreases in pulmonary function (Gong et al., 2004b, 2005, 2008; Mudway et al., 2004; Pietropaoli et al., 2004).

Pulmonary inflammation*:* CHIE studies cited in the ISA provide evidence of $PM_{2.5}$-induced pulmonary inflammation; however, the response appears to vary substantially depending on the source and composition of the PM. For example, CHIE studies of CAPs from Los Angeles did not show a significant effect on markers of airway inflammation in healthy or health-compromised adults (Gong et al., 2004a, 2004b, 2005, 2008). However, other CHIE studies conducted in Chapel Hill, North Carolina, observed significant indications of pulmonary inflammation among healthy adults following controlled exposures to CAPs (Graff et al., 2009; Samet et al., 2009). Huang et al. (2003) found the increase in BAL fluid neutrophils reported by Ghio et al. (2000) in Chapel Hill to be positively associated with the iron, selenium, and sulfate content of the particles.

Pulmonary oxidative responses: Results of CHIE studies cited in the ISA suggested that short-term exposure to $PM_{2.5}$ at near-ambient concentrations could produce mild oxidative stress in the lung. For example, Barregard et al. (2008) observed a significant increase in malondialdehyde concentrations in healthy subjects after exposure to wood smoke particles. Limited data suggest that proximal and distal lung regions might be subject to different degrees of oxidative stress during exposures to different pollutant particles (Behndig et al., 2006; Mudway et al., 2004; Schaumann et al., 2004).

Pulmonary injury: One CHIE study cited in the ISA suggests that exposure to wood smoke particles might increase markers of pulmonary injury in healthy adults (Barregard et al., 2008).

Biologic Gradient: PM CHIE Study Contributions to the Four Basic Elements of the NAAQS

PM CHIE studies at EPA have generally focused on questions related to specificity, temporality, and biologic plausibility, rather than biologic gradient. That is to say, PM CHIE studies at EPA have generally used exposures within a narrow range of PM concentrations. This is likely because CHIE study investigators have often not found consistent biomarker or physiologic responses to short-term exposures at lower PM exposure concentrations, even though large epidemiologic studies have found exposure–response associations with health outcomes at lower concentrations than those used in the CHIE studies (EPA, 2009). These differences might be partly due to increased susceptibility of population subgroups in large epidemiologic observational studies, compared to the much smaller number of subjects in CHIE

studies, and partly due to exposure mixture differences. It is possible that exposure to the complex mixtures of PM components and pollutant gases, which are so difficult to disentangle in large epidemiologic studies, has larger effects than exposure to the individual criteria pollutants within them, especially when encountered over longer time periods than the 2-hour durations of CHIE studies.

To facilitate the identification of subgroups at the greatest risk for PM-related health effects, CHIE studies have evaluated factors that contribute to the sensitivity of an individual to criteria pollutant exposures. Such studies aim to evaluate sensitivity to exposure to criteria pollutants on biomarker or physiologic responses. Based on prior knowledge, CHIE studies are designed such that clinical responses are expected to be absent or at least minimal. When studying sensitive groups, CHIE studies involve individuals who might exhibit risk factors to a small degree, but not those who are known through observational epidemiologic studies to be at considerable risk for clinical responses to criteria pollutants.

Indicators: $PM_{2.5}$ and PM_{10} are the indicators of the PM NAAQS. EPA monitors the ambient concentrations of those pollutants routinely and extensively and maintains the readings in a publicly available database. However, while pollutant gas concentrations are monitored continuously, most of the PM mass concentrations are based on 24-hour mean concentrations that are monitored only every sixth day. This severely limits their utility for studying transient short-term responses to peak exposures of PM. EPA also maintains a large, but somewhat more selective, database of monitoring values in its Air Quality System database for particle chemical constituents on an every-sixth-day schedule, and they are not used currently as indicator pollutants for NAAQS setting.[3]

Averaging time: The nation derived substantial calculable public health benefits from the implementation of the pre-2009 annual $PM_{2.5}$ NAAQS. Reductions in ambient $PM_{2.5}$ concentration were associated with substantial reductions in annual cardiovascular mortality nationwide, and especially in the northeastern United States (Laden et al., 2006; Pope et al. 2009; Thurston et al., 2013, 2016a).

No CHIE studies were cited in the ISA on health effects of long-term human exposures to PM for setting the annual average 2009 PM NAAQS for $PM_{2.5}$ and PM_{10}. Given that CHIE study exposure durations are typically a few hours, they are not capable of assessing effects of chronic exposures in humans. Instead, epidemiologic studies are used to investigate chronic effects of importance and offer results that complement the specific insights on acute effects gained through CHIE studies.

Level: The current concentration limit for the primary $PM_{2.5}$ NAAQS averaged over 24 hours is based primarily on epidemiologic studies with complementary information from CHIE. As noted above, according to the evidence considered during the PM NAAQS review completed in 2012, EPA concluded that reliance on mass concentration limits is warranted, rather than limits on concentrations of the most hazardous particle components. This circumstance reduces EPA's capacity to focus its air pollution control efforts on the various sources of the most hazardous components of ambient PM_{10} and $PM_{2.5}$. This remains an important motivator for future research studies.

Form: In the case of the PM NAAQS, the form of the standard is on a mass basis. The evidence of the adequacy of the form of the current PM NAAQS derives from the evidence of reduced mortality with reduced PM on a mass basis. This evidence accrues from epidemiologic studies, and would not be expected to come from CHIE studies.

Impacts of the Available Results of CHIE PM Studies on the PM NAAQS

As indicated above, CHIE studies have facilitated the identification of subgroups at elevated risk for PM-related health effects. By evaluating biomarker or physiologic responses to PM exposures, CHIE studies have contributed evidence for biologic plausibility of associations of PM with extrapulmonary (e.g., cardiovascular) health effects and to an understanding of factors that determine the sensitivity of an individual to PM pollutant exposures.

[3]See EPA's Air Quality System, available at https://www.epa.gov/aqs.

Contributions of a Source and Composition-Focused CHIE Study

The results of PM CHIE studies can be influenced by the composition of the PM to which subjects are exposed. The chemical composition of the $PM_{2.5}$ in diluted DE differs from that of the $PM_{2.5}$ in the ambient air. DE is greatly enriched in EC and OC, and greatly lowered in terms of toxic metals. Also, the diesel-engine exhaust composition, and the DEPs within the mixture, can vary substantially with engine type and age, duty cycle, and fuel composition, especially in terms of the relatively recent changes in the sulfur content of the fuel.

CHIE studies of DEP in the absence of other components of ambient $PM_{2.5}$ provide an example of a CHIE study with two types of potential benefits. CHIE studies of DEPs can inform EPA's decision making concerning initiatives (such as NESHAPs) to reduce PM emissions from a specific source (diesel engines). A second benefit relates to the broader research goal of linking PM composition with biologic perturbations, comparing DEP responses with those from other particle sources. Thus, while it would be anticipated that exposure–response relationships for DEPs to those of ambient-air $PM_{2.5}$ exposures would differ, the specificity of the response provides value to the PM knowledge base. However, whatever biologic perturbations that are observed in response to controlled DEP exposures could be due, at least in part, to NO_2, NO, CO, or OC gases, to condensed OC and toxic trace metals in the exhaust stream, or to their reaction products and interactions. Likewise, in comparing the results of DEP CHIE studies to inhalation exposures near roadways, another source of PM exposure in addition to the diluted tailpipe emissions is road dust resuspension, which adds PM from tire wear, brake and clutch wear, wind-blown soil, and road surface material, all of which add additional toxic trace metals and organics to the inhaled mixture.

CONCLUSIONS

CHIE studies have provided information about specificity, temporality, biologic plausibility for O_3 and PM exposure, and relevant end points. Additional information about biologic gradients and susceptible (also referred to as "at-risk") subpopulations has also been provided. The four elements of the NAAQS (indicator, averaging time, level, and form) have been influenced by CHIE study results to different degrees.

CHIE study findings also help to enrich a scientific understanding of the underlying biologic and physiologic short-term responses to daily inhalation exposures to airborne pollutants, or mixtures thereof.

- Such information is important for future NAAQS reviews and cannot be determined only through observational studies of exposures to complex ambient mixtures among groups of humans whose genetic and other constitutional variables, prior illnesses, occupational exposures, and smoking histories are not as well defined as those for CHIE study subjects.
- For complex mixtures of ambient air pollutants, a further challenge of CHIE studies and epidemiologic studies is the spatial and temporal variability of the chemical compositions of the mixtures, which makes it more difficult to assess exposure–response relationships in terms of considerations of specificity and consistency.

CHIE study results combined with information from observational epidemiologic, panel, and toxicologic studies can facilitate a holistic evaluation of the evidence and thereby provide a well-considered scientific basis for establishing or revising NAAQS.

Developing and refining biomarkers of responses to short-term inhalation exposures to specific pollutants can lead to

- Incorporation of those biomarkers in panel and cohort studies,
- Incorporation of those biomarkers in animal inhalation studies, and
- Mechanistic research to determine the utility of the biomarkers in studies of disease progression.

4

Assessment of Controlled Human Inhalation Exposure Studies at EPA and Associated Adverse Events

INTRODUCTION

Controlled human inhalation exposure (CHIE) studies at the U.S. Environmental Protection Agency's (EPA's) Human Studies Facility in Chapel Hill, North Carolina, are focused on gaining an improved understanding of short-term physiologic and biomarker responses to criteria pollutant exposures, with an emphasis on ozone (O_3) and airborne particulate matter (PM). In particular, EPA indicates that a major benefit of these studies is that they provide important information that will inform future reviews of the O_3 and PM National Ambient Air Quality Standards (NAAQS).

Nearly all of the EPA CHIE studies were conducted either in healthy young adults or in carefully selected subjects over a wider age range with predispositions to measurable transient and reversible physiologic or biomarker responses, while attempting to minimize the likelihood of adverse events.[1,2] Therefore, the objective of EPA CHIE studies has been to produce transient and reversible biomarker or physiologic responses that inform about biologic mechanisms of pollutant effects but do not cause clinical effects. The experimental results of transient outcomes (such as temporary changes in lung function) in response to controlled human exposures have contributed to EPA's Integrated Science Assessments to support reviews and revisions of the NAAQS, in conjunction with epidemiologic evidence of significant associations between ambient air concentrations of O_3 and $PM_{2.5}$ and adverse health effects, as well as with physiologic or biomarker responses reported in the epidemiologic studies (see Chapter 3). In this regard, the biomarker responses to both short- and long-term exposures to air pollutants have been critical factors in the interpretation of the roles of short-term responses in the initiation and progression of chronic effects.

Some of EPA's CHIE studies in Chapel Hill involve diluted diesel-engine exhaust (DE) that contains ambient air ultrafine particles (UFPs) composed primarily of elemental carbon (EC). The results of these CHIE studies could be more useful for informing regulatory approaches that focus on source emissions (such as National Emission Standards for Hazardous Air Pollutants for diesel-engine exhaust) as well as for revising the $PM_{2.5}$ NAAQS. Likewise, wood smoke CHIE study results could be more useful for regulatory approaches that focus on source emissions as well as for affecting the $PM_{2.5}$ or PM_{10} NAAQS. Wood smoke is a minor mass component in ambient air in most heavily populated regions of the United States, and the chemical composition of the PM within wood smoke is very different from the ambient air PM in these regions.

[1]As indicated in Chapter 2, the U.S. Department of Health and Human Services defines an adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

[2]As indicated in Chapter 1, EPA CHIE studies cannot be conducted on children or pregnant or nursing women.

THE EIGHT STUDIES IDENTIFIED BY EPA FOR CONSIDERATION BY THE COMMITTEE

Twenty-one CHIE studies had been active at EPA's Human Studies Facility at some point time from January 2009 to October 2016 (see Table C-1 in Appendix C). Eight CHIE studies were identified by EPA for consideration by the committee (see Table 4-1).[3] The committee's review of those studies (see Appendix C) included considerations of the testability of the hypotheses, appropriateness of the study design and outcome measures, and potential value of the results. The reviews are summarized below**.**

The pollutants included in the eight studies were O_3 alone in two of them, sequential exposures to O_3 alone and nitrogen dioxide (NO_2) alone in one, a mixture of O_3 plus DE in one, concentrated $PM_{2.5}$ in Chapel Hill ambient air in two, concentrated Chapel Hill ambient UFPs in one, and wood smoke in one. For the recent studies involving DE and wood smoke, the basic characterization of the exposures to air pollution mixtures in the DE and wood smoke studies was in terms of PM mass concentrations, with some data on the particle number concentration (primarily attributable to EC), as well as some characterization of the other hazardous air pollutants that were also present within the mixtures.

A common objective of the studies is to contribute to a body of knowledge about the potential health effects from exposure to air pollutants, which would add to the results of toxicologic and epidemiologic studies (see Chapter 3). It is important to note that these studies were not intended to reflect the variability of ambient pollutant concentrations in the real world, and that the relatively small number of subjects involved in each study tends to limit the generalizability of the results, by themselves, to a broader population. However, the CHIE study findings could add relevant new knowledge to future NAAQS reviews. For example, the findings of CHIE studies of $PM_{2.5}$ extracted from Chapel Hill ambient air could add new insights concerning human responses to $PM_{2.5}$ in Chapel Hill ambient air as well as possible insights to observed responses to ambient air exposures in other U.S. regions having very different $PM_{2.5}$ chemical compositions.

Cardiopulmonary Responses to Exposure to Ozone and Diesel Engine Exhaust with Moderate Exercise in Healthy Adults (DEPOZ)

Background: Assessing the components in air responsible for particular health effects is difficult because ambient air pollution is a complex mixture of gases and PM. O_3 and DE are often important components of those complex mixtures. It is stated in the Institutional Review Board (IRB) application that it is not known whether coexposure to both O_3 and DE, as would occur when humans are exposed to polluted ambient air, can induce additive or synergistic effects, and also whether exposure to DE, or DE with O_3, can alter a subsequent exposure to O_3. This study was designed to examine whether coexposures to O_3 and DE, at concentrations in the upper range of those encountered in urban settings, can induce additive or synergistic effects, and whether a previous DE exposure can alter a response to subsequent O_3 exposure.

Hypothesis: There were three specific hypotheses for this study.

- Healthy adults exposed either to DE and O_3 or DE alone on one day will not experience a significant decrement in pulmonary function in response to an O_3 exposure on the second day, relative to exposure to O_3 alone;
- A coexposure to DE and O_3 on one day would cause significant cardiopulmonary responses to an O_3 exposure on the next day; and
- Two consecutive days of O_3 exposure would affect cardiovascular responses.

[3]An application for institutional review board approval and a consent to participate in a research study for each CHIE study was provided by EPA on November 19, 2014.

TABLE 4-1 Descriptive Information on Eight CHIE Studies

Study Name (Pollutant)	Health Condition of Subjects	No. of Subjects Exposed (# male; # female)	No. of Subjects Planned to be Exposed (# male; # female)	Age of Subjects	IRB-Approved Exposure Duration	IRB-Approved Maximum Exposure Conc.	Actual Exposure Conc.
DEPOZ Diesel-engine exhaust (DE) and ozone (O_3)	Healthy	M: 11 F: 8	Study Completed	Avg: 27.8 Range: 22-53	Two 2-hour exposures: Day1: DE only, O_3 only, DE+O_3, or clean air Day 2: O_3 only	DE, 300 µg/m^3 O_3, 300 ppb	DE (µg/m^3) Avg: 295 Range: 244-326 O_3 (ppb) Avg: 300 Range: 300-300
ENDZONE [Ozone (O_3) and Nitrogen dioxide (NO_2)]	Healthy	M: 17 F: 14	Study completed	Avg: 28.9 Range: 8-41	Four 2-hour sessions: - Clean air + O_3 - NO_2 + O_3 - Clean air + NO_2 - O_3 + NO_2	O_3, 300 ppb NO_2, 500 ppb	O_3 (ppb) Avg: 300 Range: 299-300 NO_2 (ppb) Avg: 500 Range: 500-525
GEMINOZ [Ozone (O_3)]	Healthy	M: 8 F: 4	50 total	Avg: 26.7 Range: 20-36	Two 2-hour sessions: 1 for ozone and 1 for clean air	300 ppb	O_3 (ppb) Avg: 300 Range: 299-300
KINGCON [Particulate matter (PM) < 2.5 µm]	Mild asthma	M: 2 F: 14	Study Completed	Avg: 49.8 Range: 45-58	Two 2-hour exposures: 1 PM and 1 clean air exposure	Up to 600 µg/m^3	$PM_{2.5}$ (µg/m^3) Avg: 236 Range: 38-579
OMEGACON (PM < 2.5 µm)	Healthy	M: 8 F: 22	Study Completed	Avg: 57.9 Range: 51-72	Two 2-hour exposures: 1 clean air (Day 1) and 1 PM (Day 2) fish oil, olive oil, or no oil 4 wks prior	Up to 600 µg/m^3	$PM_{2.5}$ (µg/m^3) Avg: 278 Range: 83-470
SOZIAL (O_3)	Healthy; 4-point perceived stress symptom score <2 or >6	M: 13 F: 20	40 total	Avg: 27.0 Range: 21-33	Two 2-hour sessions 1 ozone and 1 clean air	300 ppb	O_3 (ppb): Avg: 300 Range: 300-300
WOODSIE [wood smoke particles (WSPs)]	Healthy	M: 17 F: 22	Study Completed	Avg: 27.5 Range: 18-38	One 2-hour session: - WSP + Live attenuated influenza virus (LAIV), or - Clean Air + LAIV	WSP, 500 µg/m^3 LAIV,1 ml FluMist	WSP (µg/m^3) Avg: 488 Range: 435-526
XCON [Ultrafine particles (UFPs)]	Metabolic syndrome	M: 13 F: 22	Study Completed	Avg: 47.3 Range: 26-70	Two 2-hour exposures: 1 (UFPs) and 1 (clean air)	Up to 600,000 UFP/cm^3	# UFP/cm^3: Avg: 211,462 Range: 17,295-563,912 UFP (µg/m^3) Avg: 118 Range: 35-359

Study Design and Outcome Measures: A randomized crossover single-blind study design was used, involving healthy subjects between the ages of 18 and 55 in four exposure regimes:

Regime 1. Combined exposure to DE and O_3 (Day 1); exposure to O_3 alone (Day 2).
Regime 2. Exposure to O_3 alone (Day 1); exposure to O_3 alone (Day 2).
Regime 3. Exposure to DE (Day 1); exposure to O_3 alone (Day 2).
Regime 4. Exposure to clean air (Day 1); exposure to O_3 alone (Day 2).

Each subject is assigned randomly to a sequence of all four exposure regimes. Each regime is separated by at least 13 days. Subjects are exposed while undergoing moderate intermittent exercise. The DE exposure concentration was 300 $\mu g/m^3$, and O_3 exposure concentration was 300 ppb. There is one follow-up visit approximately 18 hours after the last exposure.

The principal outcomes measured for pulmonary function are FEV_1 (forced expiratory volume of air that can be forcibly blown out in one second) and FVC (forced vital capacity, the volume of air that can forcibly be blown out after full inspiration). Other primary measured end points are heart rate variability (HRV), blood inflammatory factors (such as IL-6), blood clotting factors (such as fibrinogen), and susceptibility factors [such as the genotype glutathione-*S*-transferase M l (GSTM1) null].

Results: According to Madden et al. (2014), the study results suggest that the combination of O_3 and DE exposure can alter respiratory responses in a greater than additive manner, and O_3-induced pulmonary function decrements are greater with a prior exposure to DE compared to a prior exposure to filtered air.

In addition, Stiegel et al. (2015) reported that samples of blood, exhaled breath condensate, and urine collected from DEPOZ study subjects were used to develop a method for characterizing and interpreting changes in the expression of cytokines in biological media. Such changes are considered to be indicative of an inflammatory response to external stressors.

Discussion: In general, the study was designed adequately for its stated goals. The protocol described in EPA's application for IRB approval of the DEPOZ study described how the hypothesis on pulmonary responses could be tested by analyzing data collected on pulmonary function. However, the protocol to test the hypotheses concerned with cardiovascular responses is not as specific as those involving pulmonary function, and EPA's application does not state how data on cardiovascular effects would be analyzed. The sample size calculated based on statistical power considerations focused on changes in pulmonary function responses. Power for testing other responses is not clear. Thus, it is not clear whether the hypothesis involving cardiovascular responses was testable. Given the size of the expected changes in GSTM1 and the variability of responses, the sample size was probably too small to reach definitive conclusions about the effect of the GSTM1 null genotype on the effects of O_3 exposure on pulmonary function.

The comparative exposures to DE in ambient air described in EPA's application for IRB approval either were not documented or were from a simulation study, in which the exposures were much briefer than exposures of the DEPOZ study subjects.

Like all studies of limited and prescribed exposures to subjects, many questions remain. Effects upon the very young and old and upon those with existing health conditions were not investigated. The study involved a limited number of exposure conditions, none of which are typical of real-world exposures, so using the results of the study, by themselves, to predict responses in real-world situations would be limited.

The findings of the DE exposures might have marginal relevance to future reviews of the $PM_{2.5}$ NAAQS, as EC and organic carbon (OC) can represent substantial fractions of $PM_{2.5}$ mass. However, interpretations of the results are limited by the absence of knowledge of the contributions of NO_2, EC, OC, and the numerous other hazardous air pollutants within the DE mixture to the responses attributable to the exposures. Furthermore, the exhausts from newer diesel engines produce only small fractions of the hazardous air pollutants, EC, and OC compared to those emitted from diesel engines of older models (Hes-

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terberg et al., 2011). Between 1999 and 2010, total carbon (OC and EC) generally decreased in both urban and rural areas, with the strongest trends in the western States (Hand et al., 2013). OC decreased by 3.3% to 6.5% per year, while EC, which is almost all attributable to diesel engine emissions, declined by 3.2% to 7.8% per year (Blanchard et al., 2013).

Effects of Sequential Exposure to Nitrogen Dioxide and Ozone in Healthy Adult Human Volunteers (ENDZONE)

Background: As different pollutants reach peak ambient concentrations at different times during the day, it is important to consider whether exposure to one pollutant sensitizes an individual so that a response to a subsequent exposure is augmented. The purpose of this study is to determine whether exposure to O_3 or NO_2 enhances cardiopulmonary effects of healthy adults in response to a subsequent exposure to the other pollutant, relative to exposure to either pollutant without a subsequent exposure.

Hypothesis: The study is designed to test two general hypotheses.

- Preexposure to a relatively low concentration of NO_2 will sensitize individuals to a subsequent O_3 exposure and lead to greater changes in cardiopulmonary function compared to O_3 exposure preceded by clean air exposure, and
- Preexposure to O_3, at a concentration that has been previously associated with small changes in cardiopulmonary function, will prime individuals to have a greater response to NO_2 compared to preexposure to clean air.

Study Design and Outcome Measures: Healthy study subjects are involved in four exposure regimens:

Regimen 1: Exposure to clean air followed by O_3.
Regimen 2: Exposure to NO_2 followed by O_3.
Regimen 3: Exposure to O_3 followed by NO_2.
Regimen 4: Exposure to clean air followed by NO_2.

Each regimen involves exposures and intermittent, moderate exercise on two consecutive days with a third follow-up day. Each study participant is exposed randomly to all four exposure regimes, and each regimen is separated by at least 13 days. Primary outcome measures are cardiac electrophysiology, pulmonary function, and pulse-wave analysis to measure arterial stiffness. Secondary measures include analysis of blood clotting/coagulation factors and other soluble factors present in plasma.

Results: Study results were not available when this report was being prepared.

Discussion: The study was designed appropriately to meet the stated goals of the study. However, the inappropriate temporal sequence of O_3 and NO_2 exposures precludes the likelihood of effectively addressing O_3 NAAQS issues. As noted in EPA's submission for IRB approval, the real-world sequential exposures involve peak morning exposures to NO_2 followed by peak early afternoon exposures to O_3. Why then select the second of the sequential 2-hour controlled inhalation exposures 24 hours later? Exposure to NO_2, followed a day later by O_3, could be informative, although less so than would be a 2-hour delay between the two exposures on the same day, as they most often occur in ambient air. The O_3, followed a day later by NO_2, is not likely to be very informative with regard to responses to real-world exposures. Both O_3 and NO_2 exposures have been included in previous CHIE studies without clinically adverse effects. For a study of the physiologic effects of sequential exposures of inhalation exposures to O_3 and NO_2, there was little justification provided for the temporal sequences.

Epigenetic Effect Modifications with Ozone Exposure on Healthy Volunteers (GEMINOZ)

Background: Epigenetics refers to mechanisms not involving changes in DNA sequence that influence gene expression. Researchers have explored how changes in the epigenome might affect a person's susceptibility to effects caused by air pollution exposures. However, separating the role of genetics from the effect of epigenetic factors presents a substantial challenge. One approach is to study monozygote (MZ) twins, which have identical genetic sequences and different epigenomes. By involving MZ twins as study subjects, effects attributable to epigenetics can be explored separately from the effects of genetics. The study is intended to determine whether differences in baseline epigenetic profiles between subjects are associated with responsiveness to O_3 exposure and whether O_3 exposure itself causes acute changes in a subject's epigenome.

Hypothesis: Epigenetic factors in healthy individuals or individuals with the same genetic makeup (that is, identical twins) affect the responsiveness to inflammation following ozone exposure.

Study Design and Outcome Measures: Healthy MZ twins and healthy nontwin subjects are exposed during two sessions separated by an interval of about 14 days, to clean air on one day and O_3 on the other day, and involving intermittent exercise. Primary outcome measures include pulmonary function, lung inflammation, and epigenetic changes as indicated by bronchoalveoalar lavage.

Results: Study results were not available when this report was being prepared.

Discussion: This study has strong biologic justification, outcome measures are validated, and the sample size estimate is adequate for the intended power. However, the use of MZ twin studies adds another factor that may limit the applicability of the results to the general population.

Mechanisms by which Air Pollution Particles Exacerbate Asthma in Older Adults with Mild Asthma (KINGCON)

Background: As discussed in EPA's application for IRB approval of this study design, previous observational studies have indicated that asthmatics with the null genotype for GSTM1 have increased susceptibility to O_3 and DE exposures. As those previous observational studies focused on children, there remains a lack of evidence on the effects of inhaled pollutants on older adults with asthma in relation to the GSTM1 genotype.

Hypothesis: Older adults (45-65 years old) with mild asthma who have a GSTM1-null genotype will have a greater inflammatory response to PM exposure than do older adults with mild asthma who are GSTM1 sufficient.

Study Design and Outcome Measures: This study compares the response of older adults with mild asthma that are GSTM1-null and GSTM1-positive to fine particulate matter ($PM_{2.5}$) and UFPs. Subjects are randomly exposed to both clean air and concentrated $PM_{2.5}$ and UFPs, with exposures separated by a minimum of 2 weeks. Responses of primary interest include changes in FVC and FEV_1 immediately after the exposure and acute increases in airway neutrophils (as reflected in recovered bronchoalveolar lavage samples) 24 hours after exposure. Subjects are allowed to participate in the exposure study even if they decline or are excluded from bronchoscopy.

Results: Study results were not available when this report was being prepared.

Discussion: The design and methods are appropriate for the stated goals of the study. However, consideration of the relevance of the study results to all mild asthmatics needs to take into account the variability of exposures to ambient PM and ambient gaseous components (such as O_3 and NO_2).

The description of the study protocol is too long and too complicated with regard to informing potential study subjects. Because the subjects have mild asthma, discussion of risks for normal (non asthmatic) subjects is not entirely relevant. The background section of the EPA application for IRB approval reported that 736 normal nonasthmatics had a rate of <0.1% complications from bronchoscopy. The likelihood of complications for the group under study is expected to be greater. Providing the complication rates of a subpopulation with reactive airways disease would be more realistic and helpful to the participants.

Cardio-protective effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles (OMEGACON)

Background: As indicated in EPA's application for IRB approval for this study, short-term exposures to ambient PM at elevated concentrations can lead to cardiac arrhythmias, worsening heart failure, and acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk groups. Reactive oxygen species produced in humans after exposure to PM have been implicated as a potential mechanism for adverse effects of air pollutants, and genetic polymorphisms of glutathione *S*-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. Also, studies show omega-3 fatty acids have the potential to reduce cardiovascular (CV) effects, including arrhythmias, through a reduction in oxidative stress. The goal of this study was to determine if fish oils/omega-3 fatty acids would reduce or mitigate the respiratory and CV effects of PM.

Hypothesis: The study is designed to test these hypotheses.

- PM exposures cause adverse CV effects and omega-3 fatty acid supplementation pretreatment would attenuate the adverse CV effects.
- Healthy older subjects with a GSTM1-positive genotype have lower CV risk than subjects with GSTM1-null genotype when exposed to PM.

Study Design and Outcome Measures: In a randomized, double-blind study, involving older subjects (age 50-75 years), subjects are given either fish oil (containing omega-3 fatty acids) or olive oil supplements for 4 weeks. After that treatment, each subject is involved in a 2-day exposure sequence: clean air on the first day, and $PM_{2.5}$ and UFPs on the second day.

Primary outcome measures are heart rate variability measurement and peripheral venous blood markers for specific and nonspecific immune responses. Secondary measures are endothelial cell function (as measured by flow-mediated dilation of the brachial artery) and pulmonary function measurements.

The sample size is based on the potential to detect a change of 0.13 units in brachial artery diameter measured by ultrasound, as observed in an earlier pilot study conducted at the EPA facility in Chapel Hill, North Carolina.

Results: Fish oil containing omega-3 fatty acids blunted the changes in heart rate variability and QT-interval prolongation on electrocardiograms associated with PM exposure (Tong et al., 2012). Also, dietary supplementation with olive oil, but not fish oil containing omega-3 fatty acids, blunted the negative impact of PM exposure on endothelial cell function as measured by flow-mediated dilation of the brachial artery. In addition, olive oil treatment was associated with increased levels of a fibrinolysis marker (tissue-type plasminogen activator) after PM exposure.

Discussion: The strengths of this study include the biologic significance of the question of whether the omega-3 fatty acid treatment could potentially reduce the cardiac-related effects of PM exposure, and certain biologic markers of inflammation. The weaknesses include the small sample size, only one dose of

omega-3 fatty acids, and only one PM exposure, each of which can limit the generalizability to more realistic situations.

This study reinforced the biological plausibility of effects of PM on CV; thrombolytic systems observed in toxicologic and epidemiologic studies and antioxidant treatment appear to modify PM response. Additional studies would be needed to better define the antioxidant effects of omega-3 fatty acids in mitigating CV effects in sensitive individuals exposed to PM.

The Interaction of Social Factors with Air Pollution (SOZIAL)

Background: As discussed in EPA's request for IRB approval to modify the previously approved study protocol,[4] acute and chronic exposures to ambient concentrations of O_3 are associated with asthma and other health effects. Also, social factors such as psychologic stress are considered to be important contributors to asthma outcomes. A greater understanding of the effects of psychosocial stress on health responses to air-pollutant exposures would help to understand which groups and individuals are at increased risk from air pollution.

Hypothesis: Social factors such as psychologic stress modify how people respond to air pollution.

Study Design and Outcome Measures: A randomized double-blind crossover study design is used to compare the cardiopulmonary responses of two groups of healthy adults with different levels of perceived chronic stress to O_3 and clean air. Subjects who score less than 2 on the 4-point Perceived Stress Scale (PSS4) and subjects with PSS4 values greater than 6 are randomly exposed to clean air and on a separate visit to O_3, with exposures separated by a minimum of 13 days. All exposures are performed while subjects perform moderate intermittent exercise.

HRV is the primary outcome measure. Possible secondary measures include pulmonary function, analysis of blood clotting/coagulation factors, biomarkers of stress, cognitive function, pulse-wave analysis, and analysis of soluble factors present in plasma.

Results: Study results were not available when this report was being prepared.

Discussion: The design of the study is appropriate, except for some lack of clarity in the randomization procedure. Two regimes are described: one with low PSS4 and the other with high PSS4. However, it was not clear whether the randomization to ozone exposure or to clean air (placebo) was carried out within arms of PSS4 or for all volunteers together.

This study has the potential to contribute novel scientific information, largely because there have been few, if any, CHIE studies that have examined the effect of psychologic stress on O_3 exposure and biologic responses. However, other studies will have to be performed for conformation, and to explore more exactly the causal pathways involved.

Effects of Wood Smoke Particles on Influenza-Induced Nasal Inflammation in Normal Volunteers (WOODSIE)

Background: Wood smoke (from sources such as wildfires) is an important source of ambient PM. Wood fires used for indoor heating, ambience, or cooking contribute to indoor air pollution. Influenza virus infections are an important cause of morbidity and mortality in the United States and worldwide. The effects of WSP exposure on subsequent responses to infectious agents including live attenuated influenza virus (LAIV) has not been previously studied in a controlled setting. A finding that exposure to

[4]The request proposed six changes, including the addition of study personnel and an increase in the venous blood sampling amount from 25 ml to 30 ml for the study to accommodate a change in one of the proposed assays.

WSP alters influenza infection would have broad public health implications. Also, physiologic and biomarker changes, such as those considered in this study, could potentially be used for population-based studies.

This study is focused on the pathophysiology underlying the association between exposure to PM and the likelihood of a viral infection and the response to that infection. The study also is designed to test novel assays of granulocyte activation and lipid mediator activation which have not previously been used in this type of research.

Hypothesis: Exposure to WSP enhances influenza virus–induced granulocyte and natural killer cell activation, via hyaluronic acid-mediated effects on interferon gamma production. Oxidant stress and viral replication may also be affected.

Study Design and Outcome Measures: A randomized, placebo-controlled study compares nasal lavage fluid granulocyte responses to LAIV administered after either WSP or clean air, in normal healthy volunteers. Subjects receive either WSP or placebo (clean air), followed by a standardized dose of LAIV and serial postinfection sampling of nasal lavage fluids, nasal biopsy, and blood.

Results: Study results were not available when this report was being prepared.

Discussion: Strengths of the study are the public health importance of the research question, and the experimental design with a robust array of end points that is appropriate for the pathways under investigation. A strength of the design is the inclusion of repeated time points, and sampling of nasal lavage fluids, nasal biopsy material, and blood. Time points are appropriate (0, 1, 2, 7-10, and 21-28 days), covering the anticipated duration of infection and assessing acute and subacute time points. There is a clean air comparator, allowing each subject to serve as his or her own control. The study results might point to mechanisms that explain the association between PM exposure and increased risk of respiratory infection. Another strength of the study is the partnering with other laboratories, leveraging the study results to address additional end points (granulocyte activation and lipid mediator activation) that are highly relevant to the toxicologic pathways involved in this response.

Physiologic Changes in Adults with Metabolic Syndrome Exposed to Concentrated Ultrafine Chapel Hill Air Particles (XCON)

Background: Metabolic syndrome (MeS) refers to a collection of risk factors (such as high blood pressure) that increase the likelihood of developing cardiovascular disease (CVD) or type-2 diabetes mellitus (DM). As discussed by Devlin et al. (2014), clinical CVD and DM have been shown to increase the susceptibility to clinical health effects of ambient PM exposure. Also, some studies suggest that MeS might increase susceptibility to inflammatory or physiologic effects of pollution. The rationale for this study was to examine biologic responses to concentrated ambient UFP exposure in patients with MeS. Also, there is considerable interest in the potential role of ultrafine particles in causing adverse health effects, relative to $PM_{2.5}$.

Hypothesis: UFP exposure to individuals with MeS will result in changes in endothelial response as indicated by flow-mediated dilation of the brachial artery and various heart rate variability and blood biomarkers.

Study Design and Outcome Measures: This study is focused on evaluating subjects with MeS between the ages of 25 and 70 years. A double-blind study is used in which each participant is exposed to clean filtered air and air containing concentrated UFPs, in randomized order. A crossover design is used, comprising two treatments, two sequences, and two periods. Each exposure is separated by 2 weeks. Repeated measurements were taken over a 24-hour period. Outcome measures include flow-meditated dilation (brachial artery ultrasound) and heart rate variability, peripheral venous blood samples, specific and

nonspecific immune responses (cytokines and C-reactive protein), coagulation factors (von Willibrand factor, factor IX, fibrinogen, thrombin, vasoactive factors), and soluble components of PM (transitional metals).

Results: Results of this study indicate that exposure to UFPs did not cause measureable changes in brachial artery diameter or blood pressure. However, UFP exposure caused changes in cardiac electrophysiologic repolarization, heart rate variability, and vascular biomarkers of inflammation and fibrinolysis (Devlin et al., 2014). It is not clear if the findings are related to UFP size or number of particles per unit volume of air.

Discussion: The research question is well focused and the hypothesis is clear and testable. The subgroup chosen for this study is considered to be at a higher risk than the general population for CVD and DM, as well as considered to have increased risk of health problems from exposure to ambient particle pollution. Although the sample size was low, power was sufficient to test primary end points. The observed biologic and physiologic responses associated with the UFP exposures could lend biologic plausibility to observational studies finding associations of UFP with clinical outcomes. The time points for measurements following exposure, 1 and 20 hours, are sufficient to see acute and prolonged effects on CV, inflammatory, and endothelial responses.

UFP exposure caused changes in the vascular markers of inflammation and fibrinolysis and UFP exposure might affect some biologic pathways through oxidative stress processes. Most changes were observed in individuals with the enzyme GSTM1. Because the study age range of study subjects is 25-70 years and given that MeS is more prevalent in individuals older than 50 years, studies involving more subjects older than 50 years would be needed to confirm those results.

The findings of the UFP controlled-exposure studies could add new knowledge for addressing whether to establish a future NAAQS for UFPs. Such an evaluation would also need to be informed by data from studies of responses to specific component concentrations of the UFPs. Another key consideration is whether any future UFP NAAQS should rely on particle number concentration as an index of exposure, instead of a mass concentration.

EVALUATION OF EVIDENCE FOR ADVERSE EVENTS RESULTING FROM PARTICIPATION IN A CHIE STUDY

As mentioned previously, EPA's CHIE studies are not intended to induce adverse effects that would require medical intervention in study subjects. The agency strives to establish and maintain experimental conditions and involve human subjects with characteristics that reflect the potential to identify and evaluate physiologic or biologic response to pollutant exposures. The kinds of biologic responses (such as inflammation) or biomarkers considered in those studies are transient and expected to dissipate within a few days.

Consideration of risks associated with participation in CHIE studies focuses on the probability of the occurrence of a serious adverse event (such as asthma attack, myocardial infarction, or death), not the transient and reversible biomarker or physiologic responses that these short-term inhalation exposure studies are designed to examine (such as a small decrement in pulmonary function). Risks of serious adverse events temporally associated with the subject's participation in a CHIE study might be affected by one or more of the following:

- Air-pollutant exposures occurring independently from the CHIE study, several days prior to or during the multiday experimental protocols,
- Intended pollutant exposures during the experiments,
- Preexisting medical conditions or sensitivities of subjects to the CHIE study pollutant(s),
- Other experimental procedures during the CHIE study (such as blood sampling or bronchoscopy), and

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- Chance occurrences of pathophysiologic events (such as a serious adverse cardiac or pulmonary event), although unrelated to air-pollutant exposures, that might happen to subjects during the CHIE study.

To characterize risks to study participants in the eight CHIE studies reviewed by the committee, EPA compared the exposures to the various pollutants used in these eight CHIE studies to exposure scenarios associated with these pollutants that might be experienced by various groups of people living in the United States. However, those comparison scenarios are mostly unsupported by ambient monitoring data. The possibility that, in some cases, the risks in the CHIE studies could be greater than those in the comparison scenario are discussed in detail in Chapter 6.

The potential adverse outcomes are described in the protocols of the eight studies in a consistent manner, with the possible exception of DE in DEPOZ, where potential adverse outcomes are described physiologically (slight alterations in blood clotting, oxygen diffusion capacity, and changes in heart rate variability), whereas other studies describe such outcomes in terms of symptoms (such as irritation to the nose, eyes, throat, and airways; pain on deep inspiration and cough) (see Table 4-2). In all of the protocols, the expected effects are considered to be transient and reversible.

In addition to the hazards from the inhalation exposure to the pollutant, volunteers face risks posed by the bronchoscopy that is used to measure some of the effects of the exposure. Transnasal fiberoptic bronchoscopy was used in the GEMINOZ and KINGCON studies. Medical screening is designed to exclude subjects who might be at greater risk from the procedure. Also, at least one physician and several nurses are on site at all times during a CHIE study to provide emergency medical care. Subjects are removed from studies if their cardiac or lung functions deviate from expected patterns. Standard blood chemistry panels are run at various time points before, during, and after most studies. As a result EPA has experienced an overall complication rate of less than 0.1% related to bronchoscopies at the EPA Human Studies Facility Laboratory. Stahl et al. (2015) report that since the introduction of fiberoptic bronchoscopy in the 1960s, published rates of complication (for example, airway trauma, bleeding, and vomiting) have ranged from <0.1 to 11%. Mortality rates were between 0 and 0.1%.

TABLE 4-2 Potential Health Outcomes Described in EPA CHIE Study Protocols

Exposure Agent	CHIE Study	Potential Health Outcome from Exposure
Particulate Matter (Ambient Air and Diesel Engine Exhaust)	KINGCON, OMEGACON, XCON	Chest pain, mild dyspnea, headache, cough, wheeze, and decrements in pulmonary function. All of these effects are expected to resolve a few hours after exposure.
Ozone	GEMINOZ, ENDZONE, SOZIAL	Decrements in pulmonary function, irritation to nose, eyes, throat, and airways, chest pain, and cough, all of which resolve a few hours after exposure. There might be an inflammatory reaction lasting 24 hours after exposure and participants may have an increased chance of getting a respiratory infection.
Nitrogen Dioxide	ENDZONE	Decrements in pulmonary function, mild irritation to nose, eyes, throat, and airways, chest pain, and cough, all of which resolve a few hours after exposure.
Diesel Engine Exhaust	DEPOZ	Decrements in pulmonary function, slight alterations in blood clotting, pulmonary function, and changes in heart rate variability (HRV).
Wood Smoke	WOODSIE	Mild mucosal irritation to eyes and nose.[a]

[a]The consent form, dated September 24, 2014, for WOODSIE indicates that "No adverse effect on lung function or cardiovascular stability has been reported during experimental WSP exposures in humans" (page 6).

The events listed in Table 4-3 include adverse events reported to the UNC IRB for all CHIE studies conducted at the EPA Human Studies Facility Laboratory from January 2009 to February 2015 (not only the eight CHIE studies identified by EPA for consideration by the committee). The table also indicates whether the occurrence of an event led to a change in EPA's CHIE study protocol. The adverse events reports provided by EPA from four CHIE studies (ENDZONE, DEPOZ, OMEGACON, and XCON), and from an additional study (CAPTAIN), support the low short-term risk characterization for these studies. Most of the time, there were no reported incidences of the potential adverse events listed in the protocols. One subject, exposed to O_3, experienced chest discomfort on deep inspiration, and the subject was retained for monitoring until pulmonary function returned within 5% of the preexposure measurement. Another subject who had developed a persistent cough was followed up over 3 months. The follow-up activities during that period included the subject being seen by an EPA physician, receiving medication for 1 week, receiving emails and phone calls from an EPA nurse, and being scheduled for an appointment at the UNC Ambulatory Care Center Pulmonary Clinic (EPA, 2014a).

A different subject experienced an episode of bradycardia during a clean air exposure in OMEGACON. Another subject, exposed to O_3, was found to have a cardiac arrhythmia on the follow-up day.

The reported unexpected serious adverse event of paroxysmal atrial fibrillation experienced by a study subject a very short time after being exposed to concentrated ambient particles during the XCON CHIE study was appropriately noted on monitoring and, as reported in the published case report, the subject's response reverted to normal sinus rhythm spontaneously without clinical sequelae approximately 2 hours after cessation of the controlled exposure (Ghio et al., 2012). The subject was observed overnight in the hospital following the observed event.

For studies involving elderly subjects or subjects that have existing conditions, like asthma, risks might become more substantial compared to risks for younger, healthier participants. For example, in the studies involving O_3, the researchers disclose to subjects, without elaboration, that epidemiologic reports have shown that elderly people may get sick or even die in high-O_3 environments. Similarly, certain genetic characteristics might put individuals at higher or lower risk. There is a possibility that exposure to some of these pollutants could cause an asthma attack in previously undiagnosed persons or exacerbate a known asthmatic condition. Likewise, those exposures might uncover an unidentified preexisting cardiac condition or exacerbate a known condition. Both KINGCON (subjects with mild asthma) and XCON (subjects with metabolic syndrome) involve subpopulations that may already be particularly at risk. But if studies were to include more at-risk subpopulations, where EPA's experience is less likely to be predictive, the likelihood of adverse outcomes could increase. This might complicate the risk–benefit calculus in determining whether such studies, or studies that involve subpopulations with even greater risk, should be conducted. However, on the other hand, these at-risk subpopulations are likely to receive the greatest benefit from the knowledge that EPA gleans from such studies, when this knowledge is used in revising air-quality standards for the United States.

ADVERSE EVENT REPORTING

EPA defines and reports adverse events according to 2007 guidance from the Office for Human Research Protections of the Department of Health and Human Services (OHRP, 2007). EPA investigators are expected to follow the definitions and reporting time frames for adverse events and unanticipated problems provided by the IRB of record for their project (EPA, unpublished material, April 27, 2015).

UNC provides the IRB of record for CHIE studies conducted at EPA's Human Studies Facility, located on the UNC campus. UNC's Office of Human Research Ethics (OHRE) is responsible for ethical and regulatory oversight of research conducted at the university that involves human subjects, regardless of funding source. OHRE administers, supports, and guides the work of the IRBs and all related activities (UNC, 2014, pp. 31, 76).

TABLE 4-3 Events Reported to the UNC IRB for All CHIE Studies from January 2009 to February 2015

CHIE Study for Reported Event	Event Description	Reportable per IRB Policy?[a]	Corrective Action
CAPTAIN	Six subjects consented to the study using forms containing an error in the heading of a table. The heading referred to O_3 instead of PM.	Yes	Corrected and approved consent forms will be used to re-consent the six subjects. Correct forms will be provided to any new subjects.
CAPTAIN	Six subjects were quoted a higher amount of reimbursement by the recruitment office. They will be paid the quoted amount.	Yes	Given the fact that these subjects were quoted the higher amount, they will be paid $1,857 and $1,757 as specified in the consent form.
CAPTAIN	A subject was disqualified prior to scheduled exposure to PM due to an unacceptable number of preventricular contractions on overnight Holter recordings.	Yes	None needed
CAPTAIN	Subject was disqualified due to unstable blood pressure and heart rate. Subject was not exposed to PM.	No	None needed
CAPTAIN	Enrolled subject was disqualified after overnight Holter following exposure to clean air revealed cardiac rhythm findings.	No	None needed
CAPTAIN	Subject showed a 12-beat run of ectopic atrial tachycardia about 2 hours following exposure to PM.	No	None needed
CAPTAIN	Subject was disqualified following clean air exposure based on overnight Holter findings.	No	None needed
CAPTAIN	Disqualification of study subject due to illness and rescheduling difficulty.	No	None needed
DEPOZ	After 2 consecutive days of O_3 exposure, subject had a 43% and 58% decrement in FVC and FEV_1, respectively, but returned to normal by next day. This decrement normally occurs in ~3% to 5% for this age group. Chest discomfort on deep inspiration.	No	Subject retained for additional time until pulmonary function returned within 5% of preexposure number.
DEPOZ	Cardiac arrhythmia noticed on follow-up day.	Yes	Removed from study.
DEPOZ	A study participant experienced a persistent cough possibly related to participation in a research protocol at EPA's Human Studies Facility.	Yes	Subject removed from study.[b] Future subjects who present a cough within the first 15 minutes of exposure will be removed from the exposure room.
ENDZONE	Study participant received unexpected concentration of pollutant exposure. There were no adverse sequalae.	Yes	Apparently, this is the first time that an event of this nature had occurred at the EPA Human Studies Facility during several decades of CHIE studies. To prevent a similar event from occurring in the future, EPA will provide procedural reminders to exposure room operators to ensure that the NO_2 delivery system is shut down with the proper protocol.
ENDZONE	Subject removed from study after having premature ventricular contractions during exercise.	Yes	None needed

ENDZONE	Subject experienced nonsustained ventricular tachycardia during rest approximately 21 hours following clean air exposure.	No	None needed
ENDZONE	Subject felt faint during exam prior to first study-related exposure.	No	None needed
ENDZONE	Subject placed on hold due to change in blood chemistry.	Yes	None needed
OMEGACON	A study subject experienced bradycardia during clean air exposure.	No	Though IRB did not require, subject was removed from study.
OMEGACON	One study subject had high blood pressure during and 1 hour after clean air exposure.	No	Though IRB did not require, subject was removed from study.
OMEGACON	A female study subject with a history of migraine headaches had migraine after exposure to concentrated airborne particulate matter. Symptom disappeared by next day.	Yes	Protocol was modified to exclude people with history of migraine headaches.[c]
OMEGACON	A study subject had cardiac arrhythmia during clean air exposure.	Yes	Though IRB did not require, subject was removed from study.[d]
OMEGACON	Subject had a 4-beat run of ventricular tachycardia after clean air exposure. This brief arrhythmia was noted on 24-hour Holter monitor. Subject denied any symptoms.	No	Though IRB did not require, subject was removed from study.
XCON	Subject experienced atrial fibrillation/atrial flutter.	Yes	The safety protocols in place for this study worked as planned, and testing was halted early. Subject was removed from study and referred for medical follow-up of underlying condition. Incident was reported in a case report by Ghio et al. (2012).
XCON	Short episode of elevated heart rate. Subject denied any symptoms.	Yes	Subject removed from exposure room and study. Subject was provided with copies of EKG and Holter recording and referred for medical follow-up.

[a]According to EPA, this table includes adverse events and other events. Some of the events were not reportable to the UNC IRB, on the basis of its policy requirements.
[b]"Follow-up provided for 3 months after event including (1) being seen by an NHEERL physician; (2) receiving medication for 1 week; (3) emails and phone calls by an NHEERL nurse; and (4) scheduling an appointment for the study subject at the UNC. Ambulatory Care Center Pulmonary Clinic" (EPA, 2014a, p. 28).
[c]"Two days of follow-up including (1) giving the subject medicine for pain relief and a visit by NHEERL's on duty physician on the first day and (2) a follow-up conversation with the principal investigator on the second day" (EPA, 2014a, p. 28).
[d]"Two days of follow-up including review of subject's holter monitor recording a by one doctor and two nurses. On the second day, the EPA medical staff advised the study subject to see a private physician because the principal investigator believed that the study subject had an underlying medical condition. The EPA provided the study subject with a copy of medical test results" (EPA, 2014a, p. 28).
Source: EPA, unpublished material, November 23, 2015.

With the occurrence of an event deemed reportable by the UNC IRB, EPA investigators are required to report the event to various offices of EPA in addition to the IRB (EPA, unpublished material, April 27, 2015). The IRB must report to the UNC Vice Chancellor for Research unanticipated problems involving risks to subjects and others. The chancellor is responsible for all required reporting of unanticipated problems involving risks to subjects or others and the resulting IRB actions to the appropriate federal agencies. That reporting would generally be coordinated through the OHRE (UNC, 2014, p. 13).

RELATIONSHIP OF SHORT-TERM CHIE STUDY EXPOSURE TO CHRONIC DISEASE RISKS

Ambient air fine particulate matter ($PM_{2.5}$) is an important criteria pollutant because of its well-documented epidemiologic evidence for association with lifespan shortening, especially via ischemic heart disease (IHD) and for lung cancer. The epidemiologic evidence for these effects, along with evidence from other sources, was instrumental in the lowering of the $PM_{2.5}$ annual average concentration limit to 12 $\mu g/m^3$ in 2014. DE contains ultrafine EC particles and larger sized aggregate EC particles, as well as surface coatings of OC, and miners exposed to DE underground have excess lung cancer that has been associated with its EC mass concentration in some analyses (Attfield et al., 2012; Silverman et al., 2012) but to a lesser extent or not at all in other analyses (Moolgavkar et al. 2015, Crump et al. 2015, 2016). Therefore, it may be prudent to consider whether the small increments to long-term cumulative PM exposure resulting from the short-term PM exposures of the volunteer subjects in the CHIE studies present significant risk increments of chronic effects such as cancer.

One way to consider the possible magnitude of significant incremental risk is to perform a classical risk calculation. However, such a calculation would necessarily be based on results from populations exposed for variable and much longer times (years, decades) than the short exposures in CHIE studies (hours). The Committee concluded that a risk calculation for CHIE exposures based on such disparate data would be so uncertain as to be virtually meaningless, as well as potentially misleading.

Another approach is to base the risk estimate on knowledge gained in national studies of the effects of exposures to ambient air $PM_{2.5}$ and its source-related components in multiple U.S. cities. The general population is exposed to ambient air $PM_{2.5}$ that includes, as a small fraction, diesel exhaust particles (DEPs) generated by traffic sources. In the study of excess lung cancer in 100 U.S. cities associated with ambient air $PM_{2.5}$, and which involved two large national cohorts, Thurston et al. (2013, 2016a) found statistically significant associations for chronic exposures to $PM_{2.5}$ attributable to coal combustion, but only marginal associations for $PM_{2.5}$ attributable to traffic sources, and there were no such associations for any other $PM_{2.5}$ source category. The cumulative exposures to DEP in these large cohort studies were many orders of magnitude higher than those in the short-term CHIE exposures. Thus, any incremental risks for lung cancer and IHD mortality resulting from CHIE study exposures to DEP are likely to be either zero or extremely small and undetectable.

Risks of chronic diseases, such as lung cancer and ischemic heart disease, have been shown to correlate with long-term cumulative exposure to $PM_{2.5}$ (Crouse et al. 2012; Hamra et al. 2014). For several CHIE studies listed in Table 4-1, the IRB-approved maximum exposure concentrations of $PM_{2.5}$ were 600 $\mu g/m^3$ for two hours. Exposures at those concentrations would add a very small increment to the cumulative long-term ambient $PM_{2.5}$ exposures of many people in the United States. For example, the average ambient $PM_{2.5}$ concentration in the western United Sates between 2000 and 2015 (average of 58 monitoring sites) was 11.6 $\mu g/m^3$ (EPA, 2016f). Although that concentration results from a decreasing trend during those years, consider a scenario in which a person is exposed at that ambient concentration for 8 hours per day, 300 days per year, for 40 years. That would result in a cumulative exposure of 1,113,600 $\mu g/m^3$-hours, which is about 925 times greater than the potential cumulative exposure of 1,200 $\mu g/m^3$-hours for subjects in several CHIE studies in Table 4-1. This suggests that any increment of chronic-disease risk resulting from $PM_{2.5}$ CHIE exposures in the studies considered by the committee would be vanishingly small relative to real-world exposures.

The objective of EPA CHIE studies has been to produce transient and reversible biomarker or physiologic responses that inform about biologic mechanisms of pollutant effects but do not cause clinical effects. Rather, these perturbations are expected to abate over time and the experimental design typically involves monitoring subjects for a sufficiently long observation period (see Chapter 5). If the perturbations induced by the exposures were to sustain, it is uncertain how they might influence subsequent responses to ambient exposures encountered in daily life. It is the committee's judgment, however, that the types of perturbations identified in the CHIE study protocols reviewed by the committee would not persist.

CONCLUSIONS

EPA CHIE studies have been, by design, limited to exposures to subjects that are highly unlikely to exhibit responses of adverse clinical significance through

- Screening of potential subjects and
- Selection of pollutants (or mixtures), and concentrations thereof, that are not expected to produce adverse short-term responses, usually based on known associations reported in observational epidemiologic studies in larger populations, or in laboratory animals at comparable concentrations.

EPA CHIE studies are, by design, limited to the characterization only of those outcomes that reflect transient and reversible biomarker or physiologic effects, which can be used for

- Developing biomarkers of exposure, or for identifying early indicators of disease initiation and progression, and
- Studying the joint effects of different pollutants.

For the study participants in the eight EPA CHIE studies reviewed by the committee, it is the committee's judgment that any risks of a serious adverse event with long-term sequelae were unlikely large enough to be of concern, realizing it is not possible to ever conclude that there was no risk.

Specific concerns have been expressed about CHIE-study risks of chronic diseases, such as lung cancer and ischemic heart disease, which are correlated with long-term cumulative exposure to $PM_{2.5}$ (particles with an aerodynamic diameter less than or equal to 2.5 μm). However, because those diseases are considered to be associated with cumulative effects that develop over long periods, $PM_{2.5}$ exposures in CHIE studies (for example, $\leq$ 600 $\mu g/m^3$ over 2 hours) would add an extremely small increment to the cumulative lifetime $PM_{2.5}$ exposures of most people in the United States. This suggests that any increment of chronic disease risk resulting from CHIE exposures would be vanishingly small.

At this time, there is insufficient information for the committee to formulate overall determinations of the success or potential utilities of the eight CHIE studies that were provided to the committee. The committee has some concern about the adequacy of the process for ensuring the most important topics are selected for CHIE studies, and whether there was sufficient senior scientific input into that process (see Chapter 5).

5

The Continued Conduct of Controlled Human Inhalation Exposure Studies by EPA

The committee was asked to assess whether continued conduct of controlled human inhalation exposure (CHIE) studies by the U.S. Environmental Protection Agency (EPA) is warranted. To carry out that assessment, the committee evaluated past CHIE studies with respect to the requirements for determining whether a research trial is ethical (see Table 2-3 of Chapter 2). The requirements and relevant aspects considered by the committee include the following:

- *Scientific validity*: The committee considered the collective contributions of past CHIE studies to the body of scientific information as assessed systematically through the Integrated Science Assessments (ISAs) process to inform EPA's review of National Ambient Air Quality Standards (NAAQS) (see Chapter 3) and, secondarily, through peer-reviewed literature, including "state-of-the-art" medical journal reviews.
- *Fair subject selection, independent review, informed consent, and respect for potential and enrolled subjects*: The committee considered the protocols of eight recent CHIE studies, including consent forms and participant monitoring procedures (see Chapters 4 and 7).
- *Social or scientific value*: The committee considered the potential benefits to society that could result from future studies designed to address key knowledge gaps with appropriate informed consent and protection of human subjects.
- *Favorable risk–benefit ratio*: Taking into account the designs, protocols, reported adverse events, and contributions of previous CHIE studies, the committee assessed qualitatively whether the risk–benefit ratios were favorable.

In this chapter, we summarize our evaluations of scientific contributions of previous CHIE studies, societal benefits of previous studies, safety-related aspects of the study protocols, and the potential for future benefits.

SCIENTIFIC CONTRIBUTIONS OF PAST CHIE STUDIES

Review of the contributions of past CHIE studies to the ISA process is presented in detail in Chapter 3. The committee considers the ISA process to be one of the key ways EPA provides communities, individuals, businesses, and state, local, and tribal governments with access to accurate information regarding air quality and potential human health effects on the general public and sensitive subgroups. The committee also considered potential contributions of CHIE studies for informing other decision making by EPA, and to scientific understanding of biomarker and physiologic effects of ambient pollutants.

The detailed documentation of eight recently completed or ongoing CHIE studies and other materials provided by EPA (see Chapter 4) comprised adequate material for an in-depth assessment of current practices and protocols employed in CHIE studies, and current administrative and scientific review processes. The documents from the eight studies also gave some perspective on scientific priorities of EPA investigators for future CHIE studies and their rationale for these priorities. Because not all of the eight studies were completed at the time of the committee's review, and the completed studies were performed recently, the committee did not attempt to conduct a full assessment of their scientific merit, as most of the actual contributions of those eight studies to ISAs or other EPA functions would occur in the future.

To obtain a fuller understanding of the past role of CHIE studies, and potential for future contributions, the committee studied the contributions of CHIE studies conducted by EPA and other organizations to the 2009 ISA for Particulate Matter (PM) (EPA, 2009), the 2013 ISA for Ozone and Related Photochemical Oxidants (EPA, 2013), to earlier ISAs of these criteria pollutants, and to more recent professional society (such as the American Heart Association; Brook et al., 2004, 2010) scientific reviews and consequent position statements on air pollution health effects.

The committee concludes that CHIE studies have provided unique information that cannot be obtained from animal inhalation studies, or from studies of people engaged in their normal daily activities (that is, through panel studies and other epidemiologic studies). The committee also concludes that no one type of study is sufficient for developing comprehensive ISAs of ambient pollution health effects. Appropriately, EPA and its external scientific reviewers have considered multiple sources of complementary exposure–response information (that is, epidemiologic, animal toxicologic, CHIE, and *in vitro* toxicologic testing and modeling studies) as they have addressed the challenging task of reviewing and potentially revising NAAQS or carried out other decision making.

Air pollution health-effects research is iterative by nature—one discipline's results inform the development of hypotheses, designs, and methods of the other disciplines' next studies. Large observational studies have demonstrated associations between adverse health outcomes and exposures to ambient pollutants or pollution traceable to emission source categories. However, when those studies have lacked biologic data or physiologic response data to support the specificity, precise temporality, or biologic plausibility of these associations, the likelihood that the associations can be used to help estimate real health effects in the U.S. population sometimes has been challenged. Those challenges have been addressed by using complementary approaches, including animal studies and CHIE studies, with an awareness of their study design strengths and weaknesses (see the EPA ISAs). Even though the generalizability of CHIE study results is limited by the studies' narrow hypotheses and use of small numbers of study subjects, they have played a key role in evaluating and elucidating biologic or physiologic mechanisms through which air pollutants might lead to health effects (without the intent or need to observe clinical health effects from the CHIE studies).

As discussed in Chapter 3, contributions of CHIE studies to ISAs can be described by using categories adapted from the Bradford Hill considerations (Hill, 1965) that have been used in the ISA process (and by other National Academies committees) in determining the strength of the causal evidence for effects of environmental exposures.

In addition, CHIE studies have contributed to the identification of sensitive subpopulations, which helps ensure that the NAAQS are set to provide an adequate margin of safety for those subpopulations and general populations. CHIE studies also complement observational studies by demonstrating a wide range of variability among individuals in their responsiveness to exposures to different criteria pollutants.

The committee concludes from findings in ISAs and other extensive reviews of pollution health effects in the literature that, although not all CHIE studies have contributed to every one of the categories mentioned above, many CHIE studies have contributed to one or more of them. PM and O_3 studies have contributed to elucidation of specificity, temporality, plausibility, and understanding of biologic or physiologic responses of the general population and sensitive subgroups. In addition, O_3 CHIE studies have contributed to an understanding of a biologic gradient, whereas PM CHIE studies have involved the use of fairly uniform target exposure concentrations of PM mass of different sizes (coarse, fine, or ultrafine) but have not focused on clarification of dose–response relationships. This study design difference results in part from the fact that PM CHIE studies have been complicated by the variable composition of PM, which is not an issue with CHIE studies of exposure to single gaseous pollutants, such as O_3.

PAST BENEFITS TO SOCIETY

The Clean Air Act requires EPA to set two types of NAAQS: *primary* NAAQS to protect public health, and *secondary* NAAQS to protect the public welfare from known and anticipated adverse effects (such as crop damage from pollutant exposure). The law requires primary NAAQS to be set to protect

public health with an adequate margin of safety, including the health of people whose profile of risk factors increases the likelihood of adverse effects from ambient air pollution exposure. In its review of the PM NAAQS that was completed in 2012, EPA found that currently available evidence indicated that groups with increased susceptibility to PM-related health effects include children, older adults, people with heart or pulmonary disease, and people of lower socioeconomic status (EPA, 2011).

CHIE studies that focused on the review and setting of primary NAAQS have helped to define an adequate margin of safety, as required in the Clean Air Act, and have begun to define the subgroups that have elevated risk factors associated with air pollution exposures. As documented in Chapter 3 and summarized below, the committee reviewed the contributions of the CHIE studies to the O_3 and the PM presented in ISAs.

Ozone CHIE Studies

O_3 CHIE studies have been of critical importance for the ISA and the subsequent NAAQS by providing

- A basis for EPA's decision to move from a 1-hour to an 8-hour averaging time for O_3 NAAQS level (concentration).
- Demonstrations of the importance of considering susceptibility factors and variability among individuals in human physiologic and biologic responses to oxidant pollutant exposures:
  - Some participants in CHIE studies developed no change in pulmonary function while others had symptoms of cough and chest tightness and/or demonstrated up to a 30% decrease in pulmonary function after a 6.6-hour O_3 exposure, as well as symptoms of cough and chest tightness. The characteristic of responding to O_3 exposure with a decrease in pulmonary function was shown to be reproducible.
  - Some participants in CHIE studies responded to O_3 exposures with increases in biomarkers of pulmonary inflammation. These were not necessarily the same individuals who responded to O_3 exposure with a decrease in pulmonary function.
- An understanding of O_3 adaptation. For some subjects, pulmonary function responses to O_3 were reduced after repeated daily O_3 exposures, but the inflammatory response was sustained over repeated exposures.
- Evidence to support the plausibility of exposure to elevated outdoor O_3 concentrations causing increased asthma events in sensitive subgroups, such as individuals with mild asthmatic conditions.
- Biologic and physiologic evidence for O_3 effects in humans generated new hypotheses for animal studies addressing susceptibility factors with study designs for assessing the effects of O_3 inhalation on pulmonary inflammation, lung permeability, biomarkers, and particle clearance.

Particulate Matter CHIE Studies

PM CHIE studies have been valuable by providing

- Evidence for physiologic and biologic effects of exposures to elevated PM mass concentrations, suggesting biologic plausibility of epidemiologic studies demonstrating associations of ambient fine-PM mass exposures with clinical cardiovascular outcomes.
- Definition of sensitive subgroups with physiologic or biologic responses of study subjects to controlled exposures of concentrated ambient particle mass.
- Confirmation in humans of PM-related biologic or physiologic effects observed in animal toxicologic studies. Human studies and animal toxicologic inhalation studies have been complementary, in terms of understanding sources of sensitivity to PM mass effects, and in exploring organ systems biologically or physiologically perturbed by particle mass. Also, as mentioned previously,

the generalizability of those studies has been complicated by the geographically and temporally variable composition of ambient PM.

- Input for regulatory decisions concerning the adequacy of the use of a single traditional metric (that is, PM mass without regard to individual constituents of the particles) for ambient PM, which comprises mixtures that vary considerably in chemical composition and/or particle size distribution. The 2009 PM ISA indicates that many different PM constituents are linked to adverse health effects and that evidence is not yet sufficient to identify the constituents or emission sources that are more closely related to specific adverse health effects.

STUDY PARTICIPANT SAFETY

To compare the risks and societal benefits of CHIE studies, the committee considered the contributions of past CHIE studies (presented in Chapter 3 and summarized above), the level of safety afforded by the study protocols, and the likelihood of serious adverse events with any long-term sequelae (see Chapter 4). Based on those considerations, the committee judges that the risk-to-benefit comparisons for EPA's previous CHIE studies in Chapel Hill, North Carolina, have been favorable, such that the likely societal benefits are greater than the risks posed to the study participants, which are unlikely large enough to be of concern.

The short-term exposures (usually over 2 hours) to criteria pollutants used in these studies were similar to the highest ambient air concentrations that could be encountered for similar periods in the United States (EPA, unpublished material, July 14, 2005). (See Chapter 6 for a discussion of comparing CHIE study concentrations and durations to those encountered in the ambient environment.)

The biologic responses of study subjects that were anticipated by the study protocols dissipated following cessation of the exposures and were not known to have had any long-term adverse consequence to the health of any of the participants. The actual experience of participants in the studies over many years supported those assessments. For the period from January 2009 through October 2016, the CHIE studies conducted at EPA's Human Studies Facility involved 845 intentional pollutant exposures and 555 clean-air exposures. Of those exposures, one resulted in the hospitalization of a study subject, which was for overnight observation following an unexpected adverse serious event of paroxysmal atrial fibrillation a very short time after being exposed to concentrated ambient particles (see Chapter 4). The subject's response reverted to normal sinus rhythm spontaneously without clinical sequelae approximately 2 hours after cessation of the intentional exposure (see Chapter 4). The occurrence of one hospitalization over a period of almost 8 years, which corresponds to 0.1% of the pollutant exposures, illustrates that, despite substantial efforts to screen potential study subjects, some level of risk is present.

Medical Follow-Up Monitoring of CHIE Study Participants

After exposure sessions have been completed for a CHIE study, medical follow-up monitoring is often conducted as a routine part of the experiment. The length of the follow-up period is determined according to the study hypothesis and research protocol and the need to document the sequence of a transient and reversible biologic response. For example, the protocol might call for study subjects to return to the facility 24 hours after the final exposure to have blood drawn for analysis or to have their lung function assessed. When the study protocol contains no plans to obtain measurement data after the exposures have been completed and there is no indication of a reason for concern, 24 hours is often used as the point when medical staff might check on the status of the study subjects. However, the nature of the controlled exposures might warrant a follow-up period other than 24 hours.

Follow-up monitoring also is conducted in response to adverse events that might occur before the study subject leaves the facility at the end of the study or at some point afterward. Investigators have a responsibility for making certain that adverse events are treated, even after the study ends. If a person is judged to be clinically stable immediately after a study, but a symptom, such as a cough, arises afterward and then persists, the person is expected to report to the investigator.

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The Food and Drug Administration (FDA, 2009) provides a useful overview of the responsibilities of researchers who conduct clinical investigations of a drug, biologic product, or medical device. See Box 5-1. According to FDA's guidance, medical care should be provided to a subject for any adverse events related to the study until the symptom resolves. If the investigator is not able to provide the type of medical care needed, the investigator should make sure that the subject is able to obtain the necessary care from a qualified practitioner. However, the guidance does not specify the period after completion of a study when an investigator is no longer responsible for addressing a health concern that might be related to the study exposure. That decision usually is left to the judgment of the investigator to determine whether a health concern is attributable to a cause other than participation in the study or if the condition of the subject is considered to be stable.

In a response to the OIG report (see Chapter 1), EPA indicated that the following language has been added to CHIE study consent documents:

> "If the study doctor determines that your injury or illness was due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5,000. If you believe your injury or illness was due to a lack of reasonable care or negligence, you have the right to pursue legal remedy. Signing this consent form does not waive any of your legal rights."[1]

The specification of a monetary upper limit for reimbursement of medical expenses is not included in the FDA guidance (Box 5-1). Also, the 2004 NRC report on intentional human dosing had recommended that participants receive needed medical care for research-related injuries, without cost to the participants (see Box1-1 in Chapter 1). The difference between EPA's approach and the FDA guidance as well as the 2004 NRC recommendation provides a basis for EPA to reexamine its clinical follow-up responsibilities with respect to reimbursement for medical expenses.

BOX 5-1 Reasonable Medical Care Necessitated by Participation in a Clinical Trial

"During a subject's participation in a trial, the investigator (or designated subinvestigator) should ensure that reasonable medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial participation. If the investigator does not possess the expertise necessary to provide the type of medical care needed by a subject, the investigator should make sure that the subject is able to obtain the necessary care from a qualified practitioner. For example, if the study involves placement of a carotid stent by an interventional neuroradiologist and the subject suffers a cerebral stroke, the neuroradiologist should assess the clinical status of the subject and arrange for further care of the subject by a neurologist. Subjects should receive appropriate medical evaluation and treatment until resolution of any emergent condition related to the study intervention that develops during or after the course of their participation in a study, even if the follow-up period extends beyond the end of the study at the investigative site.

The investigator should also inform a subject when medical care is needed for conditions or illnesses unrelated to the study intervention or the disease or condition under study when such condition or illness is readily apparent or identified through the screening procedures and eligibility criteria for the study. For example, if the investigator determines that the subject has had an exacerbation of an existing condition unrelated to the investigational product or the disease or condition under study, the investigator should inform the subject. The subject should also be advised to seek appropriate care from the physician who was treating the illness prior to the study, if there is one, or assist the subject in obtaining needed medical care."

Source: FDA (2009, p. 7).

[1]EPA Follow Up To OIG Report No. 14-P-0154, unpublished material, submitted April 27, 2015 (page 19).

Regarding preexisting health conditions, the FDA guidance indicates the investigator should inform a subject when medical care is needed for conditions or illnesses unrelated to the study that are readily apparent or identified through the screening procedures and eligibility criteria for the study (see Box 5-1).

As part of EPA's recordkeeping of adverse events associated with CHIE studies, it would be useful for future potential study subjects to know what serious adverse events associated with participation in previous CHIE studies had occurred and how those events had been addressed.

In addition to its IRB reporting, EPA should document all serious adverse events associated with participation in CHIE studies and the actions taken in response to them. That information should be accumulated and presented to future potential participants to illustrate that a study involves risks of serious adverse events that can be anticipated and that cannot be anticipated.

Selection of Study Subjects

In assessing for biologic plausibility or sensitivity in CHIE studies, involvement of study subjects from subgroups with stable chronic conditions hypothesized to increase sensitivity to the biomarker or physiologic effects of pollution (but not to adverse events) might be more informative than involvement of healthy young adults. The committee strongly supports EPA's use of the process of medical screening of potential volunteers, thus ensuring that the health assessment is not limited to a volunteer's knowledge of his or her own health status. In addition, the committee makes the following recommendations to ensure the continuation of various important activities and initiate several new ones:

EPA should continually review and update its risk-profile information on groups that exhibit sensitivity to air-pollutant exposures to inform decisions on inclusion and exclusion criteria for the selection of CHIE study subjects.

EPA and IRBs should determine which sensitive groups are appropriate for CHIE studies, keeping in mind that appropriate expected outcomes for CHIE studies are biomarker or physiologic but not adverse outcomes.

EPA should exclude potential study participants if they are in a sensitive subgroup known to be at increased risk of a serious adverse event (such as people who have had myocardial infarction). Investigators should use up-to-date approaches (such as the use of validated and calibrated risk-stratification tools developed by the American Heart Association and the Reynolds risk score) for grouping or stratifying potential participants according to their background risk of adverse events.

EPA should ensure that, as part of its process for managerial and scientific oversight (see Chapter 2), final decisions about inclusion and exclusion criteria for the selection of study subjects are reviewed, not only internally by EPA and by IRBs but by external reviewers for each specific study.

To help reviewers to determine the appropriateness of a research-study protocol, EPA should continue the practice of registering and submitting summary results of all future CHIE studies on the website ClinicalTrials.gov.[2]

EPA investigators should continue to review the most recent current animal and human toxicologic literature and human epidemiologic literature to evaluate safety when considering fu-

[2]The benefits of registering studies and reporting results are listed at this website address: https://clinicaltrials.gov/ct2/manage-recs/background.

ture CHIE studies, with particular attention to the studies that would involve exposures to pollutant mixtures that had been included in few or no CHIE studies.

For CHIE studies involving exposures to novel pollutant combinations, EPA should evaluate the safety of the exposure concentrations by conducting dose-escalation studies that initially involve low exposure concentrations and a small number of subjects and EPA should provide sufficient time for follow-up before involving a larger number of subjects.

Communication with Potential Study Subjects and Informed Consent

After detailed review of the eight recent study protocols provided by EPA, the committee noted that there was inconsistency in approaches for explaining CHIE study risk and obtaining informed consent from potential study subjects. The committee also noted recent improvement in human subject consent forms related to EPA's ongoing CHIE studies. Chapter 7 discusses the need for continued attention to updating the methods used for obtaining consent, and applying a consistent approach across studies. It is important to provide potential participants with accrued information on the occurrence of serious adverse events associated with previous CHIE studies and the resolution of those events.

POTENTIAL FUTURE SOCIETAL BENEFITS

As indicated at the beginning of this chapter, no one study and no one type of study is adequate to satisfy EPA's regulatory needs. Epidemiologic studies have sometimes reported associations without clarity regarding specificity, temporality, or biologic plausibility. As the ISAs have concluded, well-designed CHIE studies have often provided such clarity, at least in part, despite the limited generalizability of their results. Therefore, CHIE studies can be expected to provide the future potential to assess specific biologic or physiologic responses to very well delineated exposures, particularly in sensitive subgroups. There are unresolved questions relevant to the review of air quality standards and the regulation of pollutant sources that well-designed CHIE studies could address in the future. This would include addressing important knowledge gaps in pursuit of the Clean Air Act mandate to protect public health, including the health of sensitive subgroups, with an adequate margin of safety. Addressing those gaps would enhance the potential societal benefits of future CHIE studies, for example, by providing a greater understanding of the effect of PM composition on potential health response and the mechanisms by which individuals might exhibit sensitivity to air pollution exposure.

Variability of Real-World PM Exposures

As discussed in Chapter 3, CHIE PM mass studies involve exposure to a complex mixture that varies temporally and spatially in the real world. The issue of the impact of PM chemical composition variability on human toxicity remains an important research topic that is highly relevant to EPA's regulatory tasks. If a future ISA concludes there are identifiable regional differences in PM toxicity, then future regulatory approaches that differ by region might be warranted, rather than a single, nationwide PM mass-based standard.

Do biomarker or cardiopulmonary physiologic responses to concentrated air particles vary by differing particle mass or composition or mixtures of gases? Relevant ongoing EPA CHIE studies that are evaluated in more detail in Chapter 4 include investigations of physiologic/biomarker effects of (1) ultrafine particles generated by vehicular traffic, (2) diesel-engine exhaust, (3) wood smoke, and (4) mixtures of NO_2 and O_3. However, the use of the CHIE study design to compare responses to different compositions of coarse or fine particles is challenging, as the variation in particle composition in a single location, such as Chapel Hill, is dissimilar to the particle composition in other parts of North America (see Chapter 3). While the implications of differences in PM composition for health outcomes is an active area of observational epidemiologic research, it would be impractical to use the CHIE study approach to

examine the impact of the full range of PM compositions and dose ranges on biologic perturbations associated with ambient PM exposure. **To assess the contributions of different PM components to biomarker or physiologic responses and to help target future CHIE studies, EPA should consider conducting a systematic review of CHIE studies that had been conducted in North American and other parts of the world, in which study design and participant characteristics are sufficiently similar.**

Sensitive Subgroups

Several ongoing EPA CHIE studies are seeking to identify sensitive "at-risk" subgroups and exposure responses that have not been well studied in the past. Those studies include evaluation of pollution effects on neurocognitive outcomes, the potential of wood smoke to modify biologic responses to influenza vaccine, and genomic markers and oxidative stress genotypes as sources of sensitivity to pollution. As the results of those studies are analyzed, it is important to consider what information gaps might remain that future CHIE studies might address. For example:

- Are older adults at greater risk of neurocognitive responses to air pollution?
- If air pollution influences immune response to immunization, what does that imply about the effectiveness of vaccines, the response to infection, or the timing of vaccine administration in areas with seasonal variation in air pollution levels?
- Can genomic responses to criteria pollutants or pollution from particular sources help identify sensitive individuals or biologic pathways through which air pollution influences health?

As discussed in Chapter 3, a CHIE study (Kahle et al., 2015) involving sequential 2-hour exposures to clean air and O_3 at 22°C and again at 32.5°C showed an interaction between high temperature and O_3 that may activate the fibrinolytic pathway and help to explain the adverse effect of O_3 on cardiac mortality and morbidity. That finding raises important questions concerning how temperature and pollutant exposures interact. This has relevance for warning systems (such as EPA's Air Quality Index for daily air quality) as well as regulation. Review of multiple CHIE studies with a core of harmonized physiologic or biomarker outcomes might be useful when increased statistical power is needed to assess interactions of short-term exposure effects and changes in environmental conditions.

ADDITIONAL EXTERNAL SCIENTIFIC INPUT

As discussed earlier in this chapter, several of the key considerations in determining if a CHIE study is ethical involve considerations of scientific validity and potential scientific value. While the committee concludes that EPA CHIE studies are currently addressing important questions, there are concerns about the adequacy of the process for ensuring the most important CHIE study topics are selected and the external scientific input to maximize the rigor and impact of each CHIE study. Currently, external scientific advice is provided by EPA's Clean Air Scientific Advisory Committee and its Board of Scientific Counselors through their review of the Office of Research and Development's strategic research action plans. However, those plans do not indicate specific research topics to be addressed by future CHIE studies. EPA would benefit by augmenting the process by which CHIE study topics are selected to obtain a broad range of input from the external scientific community on the importance and merit of the question being addressed, and considerations to ensure scientific validity. The external input also could help strengthen coordination of EPA's epidemiologic and toxicologic resources with those of the agency's Human Studies Laboratory to ensure that scientific progress in each area informs their future research plans in order to continue to improve understanding of participant risk factors and the potential value of the CHIE studies to be performed. The committee envisions that the input provided by the advisory panel would be nonbinding on EPA or the reviewing IRBs. However, the panel's input would be provided in advance of IRB review or internal EPA review. **EPA should convene an external scientific advisory committee of ex-**

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perts on a regular basis to review the agency's progress and provide advice on the creation of a portfolio of CHIE studies with the objective of breaking new scientific ground relevant to Clean Air Act mandates and ensuring protection of human subjects.

CONCLUSION AND RECOMMENDATION

Having evaluated the historic contributions of past CHIE studies, human-subjects study protocols, the likelihood of serious adverse events with any long-term sequelae, and the potential for societal benefits, the committee concludes that the continued conduct of EPA CHIE studies is warranted, with the improvements discussed in this chapter and in Chapters 6 and 7. Improvements are needed in (1) future human-subjects oversight, protocols, risk characterization, study subject consent processes and forms, and communications with potential participants during the informed consent process; and (2) with broader scientific oversight to maximize the potential for additional societal benefits conferred by continuing to conduct CHIE studies. Chapter 6 presents the committee's recommendations regarding characterizations of risk to inform IRBs, EPA, and potential study subjects. Chapter 7 discusses ways to improve communication with potential subjects about the study protocol, risks, and potential societal benefits.

EPA CHIE studies should continue to be undertaken cautiously under two conditions: (1) only when a CHIE study is expected to provide additional knowledge that informs policy decisions and regulation of pollutants that cannot be obtained by other means and (2) when it is reasonably foreseeable that the risks for study participants will not exceed transient and reversible biomarker or physiologic responses.

6

Characterizing Risks to Subjects in Controlled Human Inhalation Exposure Studies

The U.S. Environmental Protection Agency (EPA) has conducted controlled human inhalation exposure (CHIE) studies with the objective of producing transient and reversible biomarker or physiologic responses to inform about biologic mechanisms of pollutant effects but do not cause clinical effects. Health risks to participants cannot be assumed to be the same for each CHIE study. Risk levels will vary according to study design (such as exposure agent, concentration, and duration) and the health status or risk profile of the individual participants. In this chapter, the committee provides guidance on methods for characterizing risk levels associated with participation in CHIE studies. It is important to note that this chapter is about the risk of clinically adverse health effects, not the likelihood of transient and reversible biomarker or physiologic responses that these CHIE studies are designed to examine.

Each planned CHIE study must be approved by EPA and the Institutional Review Board (IRB) of record. EPA has the responsibility for oversight, and the approving IRB has the additional responsibility for monitoring the progress of the study and for withdrawing its approval, if necessary. One of the main considerations in deciding if a CHIE study should go forward is to assess whether reasonably foreseeable risks to the study subjects are outweighed by the utility of the study results for informing air quality management decisions (see Chapter 2). Therefore, risk characterizations are needed to weigh study-related risks and societal benefits.

AUDIENCES FOR RISK CHARACTERIZATION ASSOCIATED WITH CHIE STUDIES

There are three distinct audiences to whom risk characterization are communicated as part of designing, reviewing, and executing CHIE studies:

1. EPA Researchers. When designing the study, researchers consider the likelihood of adverse outcomes when developing and applying criteria for including or excluding potential study subjects.
2. IRB. When determining whether to approve a CHIE study, IRB members consider the risks to the participants as well as the expected benefits of the study. They also determine if sufficient safeguards (such as protocols for medical oversight) will be in place for the study subjects.
3. Potential study subjects. When deciding whether to participate in a CHIE study, individuals consider the risks associated with participating in the study. This is a critical dimension of informed consent in this process.

It is useful to further classify risks in terms of a time frame for any adverse responses that might be observed. Would a possible adverse outcome be manifest within 1 or 2 days after the exposure (an acute effect), months or years after the exposure (a chronic effect), or both? Thus, a CHIE study will require the enumeration of potential adverse outcomes of interest, the characterization of these outcomes as proximate or long term, and a decision about how risks will be communicated to the different audiences for these calculations.

EXCLUSION CRITERIA FOR SCREENING STUDY SUBJECTS

EPA researchers develop and the IRB of record approves inclusion criteria for potential study subjects, that is, specific characteristics (such as health status and age) that are required of potential subjects. In addition, exclusion criteria are developed and reviewed for precluding the involvement of potential subjects, based on risk factors (such as preexisting diseases or sensitivity to bronchoscopy or other monitoring procedures). For example, when CHIE studies seek to involve subjects with mild asthma, risk-related criteria are needed that would allow for each individual to be screened for possible acceptance into a study. Individuals who have medical conditions that increase the likelihood of adverse effects are considered to have a higher baseline risk than healthy individuals. The various exclusion criteria presented in the protocols of each of the eight CHIE studies reviewed by the committee can be classified into these general categories:

- Disease history (such as asthma, cardiovascular disease, or other acute or chronic medical condition),
- Allergies (such as allergies to chemical vapors or gases, adhesives, or pollens),
- Pregnancy status (such as positive pregnancy test, nursing),
- Medication (such as over-the-counter [OTC] anti-inflammatory agents, OTC pain medications, and Vitamins C or E),
- Prior toxicant exposures (such as being a current smoker or occupational exposure to vapors, dusts, fumes, and gases),
- Ability to exercise (such as being unable to perform required exercise),
- Medical test results (such as uncontrolled hypertension), and
- Other (such as dialysis, hepatitis B, or fainting in response to blood).

FACTORS THAT MIGHT TRIGGER AN ADVERSE OUTCOME

As indicated in Chapter 4, risks of adverse events temporally associated with a subject's participation in a CHIE study might be affected by one or more of the following:

- Air pollutant exposures occurring independently from the CHIE study, several days prior to or during the multiday experimental protocols;
- Intended pollutant exposures during the experiments;
- Preexisting medical conditions or sensitivities of subjects to the CHIE study pollutant(s);
- Other experimental procedures during the CHIE study (such as blood sampling or bronchoscopy); and
- Chance occurrences of pathophysiologic events (such as a serious adverse cardiac or pulmonary event), although unrelated to air pollutant exposures, that might happen to subjects during the CHIE study.

Ideally, inclusion/exclusion criteria will remove participants at appreciable risk of an adverse outcome as a result of preexisting medical conditions or sensitivities of subjects to the CHIE study pollutant(s). As indicated in the previous section, EPA applies a broad set of criteria when selecting study subjects. Chance occurrences of pathophysiologic events unrelated to air pollutant exposures that might happen to subjects during the CHIE study would occur at a rate corresponding to a baseline of expected responses within the general population. The risk of adverse outcomes associated with other experimental procedures used during the CHIE study (such as bronchoscopy) typically are well characterized through extensive applications in many kinds of clinical studies, and this information could be directly communicated to the IRB and the participants as part of communicating the risks associated with the conduct of the CHIE study. If an adverse event occur during a CHIE studies in which study subjects exercise, there

might be uncertainty as to whether the exercise, the pollution exposure, or the two combined brought on the event. The goal is to have exclusion criteria that reduce the likelihood that events will occur due to exercise, though not all risk factors for events will be knowable. The situation that requires additional explicit characterization of risk corresponds to the risk of adverse response associated with the study-related exposures that occur in addition to pollutant exposures in the ambient environment. The committee focused on characterization of the risk associated with air pollutant exposures during the CHIE study and preexisting medical conditions or sensitivities of subjects to the CHIE study pollutant or pollutant mixtures.

WHAT ADVERSE OUTCOMES MIGHT BE EXPECTED AND WHEN? REASONABLY FORESEEABLE RISKS

As discussed in Chapter 2, reasonable foreseeable risks refer to the likelihood of effects for which there is some credible evidence to expect they might occur as a result of participating in a CHIE study. Credible evidence might be based on epidemiologic or toxicologic studies of the CHIE study pollutant or on other information (such as effects characterized in previous CHIE studies).

Epidemiologic studies have observed that acute effects (such as cardiovascular or respiratory response) in populations associated with ambient air pollution exposures might peak on the day of exposure, after 1 day, or over multiple days following exposure. Therefore, acute effects could be expected to occur during the period from immediately after exposure to several days later.

In contrast to acute adverse effects, observed chronic effects (such as increased incidence of lung cancer and ischemic heart disease) are associated with long-term exposures to air pollution. Such effects are considered to be associated with cumulative results that develop over longer periods. In such cases, what matters most are the effects of long-term exposure, rather than the results of a short-term exposure on any particular day. As CHIE studies typically impart a very small increase in the cumulative exposure to ambient air pollution over an individual's lifetime, there is no credible evidence to suggest that chronic effects be considered among the reasonably foreseeable risks of those studies. However, because of associations between long-term exposure to air pollution and chronic effects, concerns have been expressed about whether CHIE study exposures pose an elevated risk of cancer and other chronic diseases (for example, see EPA, 2014a). Given those concerns, the likelihood of chronic effects needs to be included in informed-consent communications (see Chapter 7).

CHARACTERIZATION OF RISKS ASSOCIATED WITH CHIE POLLUTANT EXPOSURES

Once adverse outcomes are selected using the principle of reasonably foreseeable risks and some decision is made about whether these outcomes are likely to be acute or chronic, risk characterization is necessary. There are two possible methods for characterizing risks of acute and chronic effects:

- Quantitative approaches that obtain a risk estimate from a published epidemiologic study of ambient air exposures and adjusts the estimate proportionally according to the exposure duration of the CHIE study relative to the duration in the epidemiologic studies, and
- The use of an exposure scenario comparator (ESC) approach, which involves comparing experimental exposure concentrations and durations with ambient concentrations of similar magnitude and duration experienced by a population in everyday life at a certain location. For comparisons in which those characteristics are similar between experimental conditions and everyday life, risks are assumed to be the same. ESC approach examples are provided later in this chapter.

While EPA researchers and IRBs of record might seek quantitative estimates of risk of potential acute effects (for example, to estimate the increment of risk a CHIE study might add to the baseline risk associated with exposure to ambient air pollution), the committee focused on the ESC approach because it judges that approach to be the better alternative overall. That consideration is based upon the limited availabil-

ity of appropriate data for risk calculations and the large attendant uncertainty in the results (particularly uncertainty associated with estimating risks of short-term exposures from data on outdoor exposures for much longer periods of time). Also, because of the potential difficulty on the part of uninitiated individuals in understanding the implications of the quantitative results in the context of controlled short-term exposures, the committee judges that exposure comparators are more understandable than quantitative estimates of risk for the target audiences (researchers, IRB members, and potential study subjects).

The ESC approach also might serve the needs of the EPA researchers and IRBs. That approach would provide a useful context for considering chronic effects, which are considered to be associated with cumulated results of conditions developed over longer periods. In considering chronic effects, the incremental exposure of about 2 to 4 hours added by participation in a CHIE study to the cumulative ambient background exposure over the life of an individual would be extremely small and calculating a risk estimate would involve too much uncertainty, such that the risk estimate would have little meaning. Therefore, the committee strongly prefers the use of the ESC approach for the characterization of risks related to CHIE study participation.

USE OF THE EXPOSURE COMPARATOR APPROACH FOR CHARACTERIZING RISK

Use of comparative scenarios is most appropriate if the risk to the comparative population is likely to be higher than the risk associated with exposures in a CHIE study. Such a comparison involves the use of an ambient exposure concentration that is higher than the exposure concentration in the CHIE study and an ambient concentration duration in the comparative scenario that is at least as long as the experimental exposure duration. It is not advisable to compare a lower ambient concentration over a longer period compared to the CHIE study exposure concentration and duration. Attempting to represent an equivalent ambient exposure in this way introduces more uncertainty as to whether the risk in the comparative scenario is actually greater than that in the CHIE study.

The first step in developing a comparative exposure scenario involves identifying a population that is (or was) in a location with a higher ambient concentration for a longer period of time compared to the CHIE study experimental conditions. Insofar as possible, the comparative scenario ought to be one that the participants in the CHIE study can readily identify with and understand. However, recent improvements in U.S. air quality might make it difficult to find recent ambient concentrations that are appropriate for use in an exposure scenario. Instead, the scenario could include a population at a U.S. location in the past, or a present population in another country. Also, if a particular exposure regimen has been used numerous times in previous CHIE studies, without the occurrence of adverse effects, the accrued experience from those studies could be useful in developing a reasonable exposure comparator. We provide examples of comparative scenarios for exposure to diesel exhaust and $PM_{2.5}$.

Example: Comparative Exposure Scenario for Diesel Exhaust

Coble et al. (2010) report on a survey conducted between 1998 and 2001 of exposures to diesel-engine exhaust particles (DEPs), quantified as the airborne concentration of respirable elemental carbon, in seven underground mines in the United States in which diesel equipment was used. One of the mines (for limestone) had very little exhaust ventilation. In this mine, personal exposures to DEPs over a full shift (8 hours) for eight underground jobs (based on 97 personal samples) averaged between 313 and 488 $\mu g/m^3$ (Coble et al., 2010, Table 4). By comparison, the participants in the DEPOZ study were exposed to 300 $\mu g/m^3$ of DEPs for a total of 4 hours. Therefore, these miners were exposed over extended periods to higher concentrations of DEPs throughout each 8-hour shift than the participants in DEPOZ who also were exposed for only one or several days during a shorter period (4 hours). The researchers did not evaluate the health responses of the miners to the measured exposures.

Example: ESC Approach for $PM_{2.5}$

Ambient concentrations in the United States provide relevant data for exposure comparator scenarios. Table 6-1 presents the highest $PM_{2.5}$ 1-hour concentrations recorded during 2014-2015 from EPA's AirData website[1] and the corresponding 2-hour and 4-hour average concentrations from monitors designated for making National Ambient Air Quality Standards (NAAQS) compliance decisions. During that time there were 14 episodes recorded in the United States in which the 2-hour average $PM_{2.5}$ concentration exceeded 300 $\mu g/m^3$, and two episodes in which the 2-hour average $PM_{2.5}$ concentrations exceeded 600 $\mu g/m^3$. There were 9 episodes recorded in which the 4-hour average $PM_{2.5}$ concentration exceeded 300 $\mu g/m^3$.

The U.S. Department of State conducts hour-by-hour PM air monitoring at the U.S. Embassy in Beijing, China, and at four U.S. consulates in China (U.S. Department of State, 2016).[2] At the embassy in Beijing, 1,920 episodes were recorded between 2008 and 2015 in which air concentrations of $PM_{2.5}$ remained greater than 300 $\mu g/m^3$ for 2 consecutive hours, with the peak 2-hour average concentration reaching as high as 983 $\mu g/m^3$. During the same period there were 32 episodes in which air concentrations of $PM_{2.5}$ remained greater than 600 $\mu g/m^3$ for 2 consecutive hours.

In the KINGCON CHIE study, $PM_{2.5}$ exposure durations were 2 hours and the concentration range was 38 to 579 $\mu g/m^3$ (see Table 4-1 in Chapter 4). A person, who happened to remain outside during one of the 1,920 episodes in Beijing near to where the ambient measurements were taken, would have been exposed to a higher concentration of $PM_{2.5}$ than were participants in KINGCON. The KINGCON application for IRB approval called for terminating exposure if the PM concentration exceeded 600 $\mu g/m^3$ for 6 minutes, although confirmation could possibly take another 10 minutes. However, it is clear from the above that, even if that occurred, there were episodes in Beijing that lasted longer and with higher concentrations. Also we see from Table 6-1 that occasionally there have been exposure episodes in the United States that lasted longer and were at higher concentrations.

The OMEGACON CHIE study called for exposing participants for 2 hours to Chapel Hill airborne PM up to a maximum concentration of 600 $\mu g/m^3$. Using the same terminating procedure as in KINGCON when the exposure concentration exceeded 600 $\mu g/m^3$, actual achieved concentrations were as high as 470 $\mu g/m^3$.

Thus, the highest possible 2-hour exposure concentrations of $PM_{2.5}$ in the OMEGACON and KINGCON CHIE studies were exceeded numerous times recently in Beijing. The $PM_{2.5}$ concentrations in the OMEGACON and KINGCON CHIE studies also were exceeded occasionally at various locations in the United States.

Evaluation of Exposure Comparisons Used in EPA CHIE Studies to Put Risks in Perspective

Each of the CHIE studies reviewed involved exposure to O_3, ambient $PM_{2.5}$, DEPs, or their mixtures. In addition, ENDZONE involved exposure to NO_2. In each IRB application, the planned exposures to these substances were placed in perspective by comparing them to exposures to the same substances in other situations. Quotations from the IRB applications regarding these comparisons are presented in Table 6-2. Many of those comparisons also are presented in the consent forms for potential study subjects.

[1]EPA's AirData website is at https://www3.epa.gov/airdata/.

[2]Only data labeled "verified" were used in these calculations. However, the website states that these data might not be "fully verified or validated."

TABLE 6-1 Largest Concentrations in 1-Hour $PM_{2.5}$ samples in 2014 and 2015 in EPA Air Data with Corresponding 2- and 4-Hour Average Concentrations

State	County	Year	Day	Hour of Day	Sample Value ($\mu g/m^3$)	2-Hour Average ($\mu g/m^3$)	4-Hour Average ($\mu g/m^3$)
Oklahoma	Love	2015	8-Sep	18	617	439	309
Washington	Snohomish	2015	4-Jul	23	512	512	481
California	Imperial	2015	1-Jan	1	448	432	357
Washington	Pierce	2015	4-Jul	22	448	366	271
California	Ventura	2015	4-Jul	20	427	235	133
Indiana	Allen	2015	5-Jul	0	406	358	287
South Dakota	Custer	2015	13-Apr	20	348	325	216
Arizona	Maricopa	2014	1-Jan	0	1167	921	683
California	Inyo	2014	30-Jul	20	955	711	396
Hawaii	Maui	2014	3-Sep	14	798	568	289
Alaska	Kenai Peninsula	2014	26-May	5	589	568	516
Kentucky	Jefferson	2014	5-Jul	21	542	355	220
California	Placer	2014	23-Sep	7	500	484	454
Arizona	Santa Cruz	2014	1-Jan	2	425	423	411
Arizona	Santa Cruz	2014	24-Dec	14	425	387	320

TABLE 6-2 Exposure Comparisons Used in EPA CHIE Studies

Study	Exposure Regime	Exposure Comparisons Used
DEPOZ	2-hour exposures to 0.3 ppm O_3 and/or 300 $\mu g/m^3$ DEP	"Occupational levels [concentrations] for some truck drivers are generally about 100-300 $\mu g/m^3$, and average 900 $\mu g/m^3$ for some [underground] mines where diesel powered machinery is used. A recent study demonstrated DEP concentrations during drive-by incidents averaged about 125 and 199 $\mu g/m^3$ at the height of an adult pedestrian and a child in a stroller, respectively. Using a 2006 diesel engine (generally recognized as emitting less PM mass than most older models currently on the road), it was demonstrated that an average DEP concentration up to 364 $\mu g/m^3$ (over ~9 sec) could be generated at near roadside monitoring stations at head level during drive by simulations with a peak concentration of [DEP at] 860 $\mu g/m^3$ [Buzzard et al. 2009]."
KINGCON	2-hour exposures to $\leq$ 300 $\mu g/m^3$ $PM_{2.5}$ or UFP	"The particle burden, on a mass basis, will not exceed an exposure an individual receives over a 24 hour period while visiting a typical [US] urban center on a smoggy day."
OMEGACON	Study plan: 2-hour exposures to $\leq$ 600 $\mu g/m^3$ $PM_{2.5}$ or UFP	"The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical [US] urban environment on a smoggy day."
ENDZONE	2-hour exposures to 500 ppb NO_2 and/or 300 ppb O_3	"Additionally, the total amount of O_3 that study participants will be exposed to during the two-hour period is equivalent to what they would be exposed to in a city at the current eight-hour NAAQS. The NAAQS for O_3 when the study was initiated was 75 ppb for an eight-hour period, which represented a cumulative exposure to 600 ppb O_3 during the eight-hour period. This is equivalent to a two-hour exposure to 300 ppb (cumulative exposure = 600 ppb [sic]), as will be done in this study." "Previous controlled human exposure studies have utilized NO_2 concentrations equal to or higher (up to 2000 ppb) than those that will be used in this study. Additionally, the NO_2 levels [concentrations] that will be used in this study are lower than those that have been measured around an [unvented] operating gas stoves [Goldstein et al. 1988; Leanderer et al. 1984].
SOZIAL	2-hour exposures to 300 ppb O_3	"Additionally, the total amount of O_3 that study participants will be exposed to during the two-hour period is equivalent to what they would be exposed to in a city at the current eight-hour NAAQS. The [then] current NAAQS for ozone was 75 ppb for an eight-hour period, which resulted in a cumulative exposure to 600 ppb [sic] O_3 during the eight-hour period. This is equivalent to a two-hour exposure to 300 ppb (cumulative exposure = 600 ppb-hr), as will be done in this study."
WOODSIE	2-hour exposures to 500 $\mu g/m^3$ wood smoke PM	"The wood smoke PM exposure in this study (500 ug/m^3 for 2 hours) is lower than that routinely encountered by forest firefighters, people living in areas near forest fires or agricultural burning, or people in developing nations who use biomass fuels for cooking. It is similar to concentrations encountered routinely indoors in homes that use wood for heat, or outdoors over the course of a day in cities where wood is commonly used for heating fuel in the winter. While the precise risk is unclear, a single 2-hour exposure at these concentrations is unlikely to pose more than minimal risk."
XCON	2-hour exposure to $\leq$600,000 particles/cc of concentrated UFP from ambient Chapel Hill air (predicted average of 110,000–330,000 particles/cc)	"We will establish 600,000 particles/cc as a maximum, which is less than or equivalent to what people would inhale while driving along a heavily traveled highways [sic] in a city such as Los Angeles."
GEMINOZ	2-hour exposures to 300 ppb O_3	"[T]he total amount of O_3 that study participants will be exposed to during the two-hour period of this study is no greater than that allowed by the [then] current 8 hour NAAQS for O_3 of 0.076 ppm."

Each of the studies that involved O_3 exposure (DEPOZ, ENDZONE, SOZIAL, and GEMINOZ) exposed the volunteers to 300 ppb for 2 hours. Except for DEPOZ, which provided no basis for exposing volunteers to 300 ppb O_3, each of these studies compared the cumulative exposure from the planned 2-hour O_3 exposure (300 ppb over 2 hours or 600 ppb-hours) to the cumulative exposure resulting from 8 hours of exposure to O_3 allowed by the 8-hour NAAQS of 75 ppb (also 600 ppb-hours). (ENDZONE and SOZIAL incorrectly referred to this exposure as simply 600 ppb.) But a given cumulative exposure over 2 hours might possibly be more damaging than the same cumulative exposure spread out over 8 hours (see discussion below). Therefore, this comparison is of questionable relevance.

The studies that involved PM exposure (DEPOZ, KINGCON, OMEGACON, WOODSIE, and XCON) compared the planned PM exposure concentration to concentrations that could occur in different situations or environments. However, they involved exposures to PM mixtures that varied in particle size distribution and chemical composition, and only one of the studies, DEPOZ, had a reference for the comparison exposure scenario.

In DEPOZ the claims that DEP exposures of some truck drivers are generally about 100-300 $\mu g/m^3$ and average 900 $\mu g/m^3$ for some underground mines were not referenced. A recent assessment of DEP exposures in seven mines with diesel equipment showed much lower concentrations than 900 $\mu g/m^3$ (Coble et al., 2010). The documented comparison in DEPOZ with exposures near roadways (Buzzard et al., 2009) was from a simulation study rather than from monitoring of DEP concentrations near roadways. The reported average DEP concentration of 364 $\mu g/m^3$ was the highest of 10 measurements and it lasted only 9 seconds. The reported peak concentration of 860 $\mu g/m^3$ was from a "typical acceleration test" in which DEP concentrations greater than 500 $\mu g/m^3$ lasted only about 1 second. The committee considers it inappropriate to compare these very brief exposure situations, lasting only a few seconds, under simulated conditions, to the 2 hours of exposure to DEP planned for the DEPOZ study.

KINGCON and OMEGACON both state that exposures to PM in these studies will not exceed what would be encountered over 24 hours in a typical urban environment on a smoggy day. No reference is provided for this comparison. Because that statement might not be true in all cases, it would be much better to specify the U.S. urban centers where those concentrations were observed. Even if validated, a given cumulative exposure spread out over 24 hours might not involve the same risk as the same cumulative exposure occurring over only 2 hours.

WOODSIE compared the planned exposure concentration of 500 $\mu g/m^3$ wood smoke PM to a number of exposure situations, including forest firefighters, people who use biomass fuels for cooking, people indoors in houses heated by wood burning, and people out of doors in cities where wood is used for heating. None of these comparisons were documented. Moreover, some of them seem inappropriate. Risky exposures to forest firefighters could be justified because they have accepted a high-risk societal responsibility to protect life and property. Also, they are likely to wear protective respirators at times to reduce personal exposures.

XCON compared CHIE exposures to exposures while driving along a heavily traveled highway in a city, such as Los Angeles, although this comparison was not documented. XCON also mentions an ongoing study that on some days exposed participants to concentrations as high as 1,181,000 UFP/cc with no symptoms of discomfort or clinically relevant responses, and a study in the Chapel Hill facility that exposed participants to 1 to 3 million UFP/cc with no clinically relevant responses. However, the durations of exposures in these studies were not mentioned. Also cited is Shah et al. (2008) (referred to as Frampton, 2008), which "exposed volunteers for several years to ultrafine [elemental] carbon particle concentrations of 10 million particles/cc and have not reported any clinically relevant changes." However, "several years" apparently refers to the total time this group has been conducting these studies, as the exposures in the cited article lasted only 2 hours. Also, the Shah et al. particles had a median diameter of 27.9 ± 2.2 nm, whereas the instrument used to concentrate particles in XCON produced particles with diameters in the range 30-250 nm. Thus, the particles in the Shah et al. study were generally smaller than those in XCON and consequently could pose different levels of risk.

ENDZONE involved exposure to 500 ppb NO_2 in addition to the 300 ppb O_3 exposure. It is stated that previous human exposure studies have utilized higher concentrations of NO_2, but this statement was

not documented. It is further stated that the planned NO_2 exposures in this study were to be lower than have been measured around operating gas stoves. Two references were provided to support this statement (Goldstein et al., 1988; Leaderer et al., 1984). Leaderer et al. (1984) reported on NO_2 in houses with kerosene space heaters, not gas stoves. Furthermore, this reference was to an abstract that contained no information about NO_2 concentrations. A later publication by the same authors (Leaderer et al., 1986) apparently reported on the same study, and the highest reported average NO_2 concentration in 25 combinations, defined by location within house and number of kerosene heaters and gas stoves, was less than 50 ppb NO_2. Goldstein et al. reported on lung function versus NO_2 concentrations while cooking with a gas stove. This paper contains graphs showing roughly 200 highest average 5-minute NO_2 measurements, most of which are less than 200 ppb and only 5 appear to be greater than 500 ppb. The information provided on the ENDZONE consent form seems to imply that participants could expect to experience higher exposures to NO_2 around an operating gas stove than they will experience in the study. This is not consistent with the information provided in the two studies cited in ENDZONE.

In 2010 EPA promulgated, for the first time, a 1-hour NAAQS for NO_2 (100 ppb) (75 Fed. Reg. 6474 [2010]). Thus, NO_2 exposures planned for ENDZONE (500 ppb for 2 hours) exceeded this standard by a factor of 5. It is inconsistent to compare the planned exposure to O_3 to the O_3 NAAQS, while ignoring the fact that in the same study the planned exposure to NO_2 is five times higher than the 2010 NO_2 NAAQS.

As the above review indicates, most of the comparison exposure scenarios in these CHIE studies were inadequate. Many were undocumented, and those that were documented were not all appropriate.

Exposure regimes that produce the same cumulative exposure may not produce comparable effects, even if the exposure length is only a few hours. For example, exposures to variable O_3 concentrations over 6-8 hours can elicit somewhat larger decrements in forced expiratory volume for 1 second (FEV_1) than a constant exposure over the same time period even if the cumulative exposures are equal (Hazucha and Lefohn, 2007). Thus, the practice used in the IRB applications of comparing a 2-hour exposure to O_3 to an 8-hour constant exposure at the 8-hour NAAQS might not be a valid comparison even if the cumulative exposures are comparable. Similar situations might occur for exposure to PM and other air contaminants. Thus, the most valid comparisons to planned exposures in human exposure studies are comparisons to populations exposed to comparable or higher concentrations for comparable or longer times.

RECOMMENDATIONS

The committee recommends that risk-characterization objectives be addressed by using an ESC approach in which the risk associated with a CHIE study exposure is likely to be lower than the risk to the comparative population.

To illustrate that the risk associated with the participation in a CHIE study is likely lower that the risk to the comparative population, the comparative scenario involves a documented ambient exposure concentration that is higher than the exposure concentration in the CHIE study and an exposure duration in the comparative scenario that is at least as long as the experimental exposure duration.

The comparative exposure scenarios should be documented fully so that the reasonableness of the comparison can be evaluated. Comparative exposure scenarios should be based on populations in the United States, insofar as possible. When that is not feasible, scenarios involving populations with demographics and life styles as similar as possible to those in the United States should be given precedence.

In planning a CHIE study, EPA should obtain the appropriate monitoring data for exposure comparator scenarios using locations where populations are or were exposed to ambient concentrations exceeding the exposure concentration envisioned for the CHIE study. When developing such comparator scenarios, differences between the CHIE study subjects and the comparative population (for example, regarding health and susceptibility) should be considered. In addition consideration should be given to the reliability of the ambient exposure monitoring data available for the

comparison (for example, data obtained from personal monitors, fixed-site monitors, or geospatial estimates) and the potential inaccuracies in personal exposure estimates that can result from use of monitored ambient concentrations.

Such considerations in comparing study subjects with specific populations and experimental exposures with ambient pollutant concentrations also would be required in attempting to develop quantitative risk estimates. Alternatively, if a particular exposure regimen has been used numerous times in previous CHIE studies, without the occurrence of adverse effects, the accrued experience from those studies could be useful in developing a reasonable exposure comparator.

It is important to note that if a CHIE study is being proposed for which no appropriate ambient concentrations (past or present) can be found, and no previous CHIE studies without adverse effects are applicable, it might be an indication that the CHIE study requires further explicit justification or should not be conducted.

For communication with study subjects, risk should be characterized in a descriptive and comparative manner using an ESC approach. This should be useful in explaining the type of exposure that will be similar to exposure as part of the study. These should be evidence based with an explanation of how they were developed. Comparison should be accessible and familiar (see Chapter 7 for more detail). Consistent expression of these complex concepts for individuals with limited health numeracy is essential.

7

Communication about Informed Consent in Controlled Human Inhalation Exposure Studies

INTRODUCTION TO INFORMED CONSENT AND THE COMMON RULE

Chapter 6 introduced the concept of exposure comparators and recommended that, for communication with Institutional Review Boards (IRBs) and study participants, risk should be characterized using exposure comparators. These comparators should be useful in explaining and putting into perspective the exposures planned for the study. Chapter 6 also explains how the comparators were developed. This chapter expands upon Chapter 6, and focuses specifically on the communication of risk to study participants through the informed-consent process. It also provides recommendations regarding the content of consent documents and the assessment of participants' understanding of informed consent.

As discussed in Chapter 2, the ethical principles governing informed consent have been forged over many decades in response to abuses to human subjects who participated in experiments when consent was either entirely absent or deeply compromised (Faden et al., 1986). The Belmont Report, describing the basic principles widely accepted as the undergirding ethical norms for The Common Rule, lists "respect for persons" as its first principle. This principle "incorporates at least two ethical convictions: 1) that individuals should be treated as autonomous agents; and 2) that persons with diminished autonomy are entitled to protection" (NCPHSBBR 1979). Thus, informed consent has, as its basic aim, the protection and enhancement of the autonomy of research participants. It is composed of three essential elements: (1) *a disclosure process* provided by investigators to an individual capable of making a decision that is free of duress or coercion, (2) *a deliberation process* between researchers and potential participants to ensure understanding, and (3) *a decision* to participate or not, grounded in the prospective participants' values.

In fulfilling the disclosure requirement, *the Common Rule* enumerates important items to be disclosed. Some of the most important areas of disclosure are (1) the nature and purpose of the research, (2) the procedures to be followed in the protocol, (3) "reasonably foreseeable risks or discomforts," and (4) any potential benefit to participants [45 CFR 46] (NRC, 2004a). "Reasonably foreseeable risks" are not further defined in the regulations and can therefore be a source of ambiguity and misunderstanding. This report has provided further guidance on this point in Chapter 2, as well as later in this chapter.

The deliberation process requires study personnel to not only inform, but to *actively enable* potential participants to "deliberate effectively" about risks and benefits, and to understand the social aims of the research (King, 2005). Facilitating such effective deliberation is often not simple and is fraught with pitfalls. Participant deficits in scientific literacy, and risk and benefit miscalculation, often confounded by poor understanding of probability and other numeric information, can be major barriers to a valid consent. The committee's view is that expressing risk using exposure comparators (see Chapter 6) will mitigate the difficulties in understanding risks due to poor numeric comprehension, and also the concerns that numerical estimates of risk might be uncertain (Pleasant et al., 2016). To help with this task, this chapter contains recommendations about effective communication with study participants.

While there are a very few trials with human subjects in which consent is not mandatory (Emanuel et al., 2000), for the vast majority of studies informed consent is understood as a *sine qua non* for ethically and legally permissible research. While not sufficient by itself, consent is absolutely necessary. This is

especially true for controlled human inhalation exposure (CHIE) studies involving volunteers, and for which societal benefits are the overriding aim, with no personal medical benefits for participants other than previously unrecognized conditions being discovered in the medical screening process. The following sections describing risks and benefits are critical to this chapter's focus on communication, given the importance of clear communication of potential risks and benefits during the disclosure process element of informed consent for the CHIE studies.

Risks

Risk characterizations are an especially important part of disclosure to potential participants. Every effort is needed to ensure that these characterizations are accurate, scientifically grounded, intelligible to people, and inclusive of a discussion of "reasonably foreseeable risks," as required by the Common Rule (see Chapter 2):

- *Reasonably foreseeable risk disclosure* is a requirement of the Common Rule, but it does not include all possible risks. An overly detailed list of all possibilities can result in a less valid consent process, since it groups the anticipated or likely risks with those that are only distant possibilities. The result is not clarity or decisional enablement, but confusion (Resnik, 2013; Rid et al., 2010). So while not all possible risks belong in a consent disclosure process, inclusion of risks that are potentially remote but of great magnitude should be considered part of a complete disclosure (King, 2000). According to Resnik (2013), a risk is reasonably foreseeable if there is credible evidence to expect that a harm might occur. Evidence for risks might be obtained from empirical research, past experience, or scientific or mathematical principles.
- *Addressing risks of concern to participants* is also part of a valid consent. Allowing people to judge risks for themselves and determine if they are willing to assume those risks is essential in respecting the autonomy of participants. In addition, answering all questions of participants, even those concerning the most improbable risks and hypothetical possibilities, is necessary for a valid informed consent.
- *The use of "real-life" exposure comparisons in risk descriptions* might be conducive to participant understanding but needs an evidentiary or expert opinion foundation to be credible. For example, phrases such as "You could potentially inhale a similar amount if you visited a large city, such as Los Angeles, New York, or Mexico City on a smoggy day" need to have such backing to avoid misunderstanding. In addition, communication of numeric risk estimates (such as extra risk of 1 in 100,000 exposed) can be particularly challenging and necessitates quantitative literacy skills of both researcher and the study participant. Participants' perceptions might influence their beliefs and understanding of these comparisons and these perceptions need to be acknowledged and discussed.

Benefits

The Common Rule states that risks to subjects must be "reasonable in relation to anticipated benefits" to research subjects, and in relation to the importance of the potential knowledge to be gained from the research [40 CFR 26.111(a)(2)]. The Belmont Report added another obligation: "to give forethought to the maximization of benefits and the reduction of risk that might occur from the research investigation" (NCPHSBBR 1979). Beyond these statements, little guidance about benefit disclosure was provided. Most codes of research ethics were designed with research on new medical therapies and the protection of patient-subjects in mind. The Nuremberg Code is focused on nonpatient subjects, but it was silent about subject benefits.

The CHIE studies conducted by the U.S. Environmental Protection Agency (EPA) involve human-subjects research of a nonmedical nature that exposes volunteers, with the ultimate goal of producing knowledge to shape sound environmental standards. Because of these differences, a clear delineation of

the types of benefits relevant to EPA research is called for. This taxonomy builds upon and expands the categorization of benefits in NRC (2004a) as "societal" and "personal."

Societal Benefits

Societal benefits are the overriding purpose of all research, but they play an especially prominent role in EPA CHIE studies. In this context, societal benefit can mean better health for specific populations or for society generally if the results lead to improved health policy. However, societal benefit is always an aspiration, a hoped-for outcome, rather than a certainty. The CHIE studies conducted by EPA offer no medical benefits to exposed participants and, therefore, are justified solely by the value of the knowledge to be gained from the research.

Even though societal benefits provide the overriding ethical justification for EPA studies, potential volunteers typically see benefits accruing to them personally as relevant to their decision to participate.

Personal Benefits

- *Medical benefits to research participants*: Multiple studies have shown that research participants, especially those who are ill, typically overestimate the potential for direct medical benefits for themselves from participation. Some individuals also fail to differentiate research participation from routine clinical care, and thereby labor under a "therapeutic misconception" (Henderson et al., 2007). This is not a major hazard for studies involving healthy volunteers. Yet, even with healthy volunteers, there is potential for misunderstanding if physical exams required for enrollment eligibility are construed by investigators or participants as a medical benefit. Such inclusion/exclusion exams are not a medical benefit because they are not part of a doctor–patient relationship in which the patient's well-being, rather than scientific knowledge, is the primary aim. Such eligibility exams may, however, result in information of value for individual participants.
- *Health information of value to participants*: Either in the exams involved for enrollment eligibility or in the required activities of research protocols, participants may learn something of value with regard to their health status. For example, they may become aware of hypertension or a cardiac problem that needs medical attention. Such health benefits may or may not accrue, and are in any case fortuitous and not an objective of inclusion/exclusion procedures. Descriptions of such procedures need to be carefully distinguished from medical examinations or therapeutic interventions. For example, model benefit language for distinguishing medical benefits from health information of value to the participant in EPA CHIE studies might include the following:

  There will be no personal medical benefits to you from participating in this study. You will receive a health exam [include details] at no charge to you, but this exam is to determine your suitability for participation in the research, and is not equivalent to a medical exam given by your doctor.

- *Benefits of altruism*: Research participants can reap psychologic benefit from knowing that they contribute to scientific progress. In some understandings of research ethics, the altruistic participant is viewed as the most desirable. Altruistic individuals may see participation as a moral obligation of citizenship, as a gift to science or humankind, as a religious responsibility, or in a variety of other ways. Altruistic actions are considered virtuous because they are undertaken without expectation of reciprocity or recognition, and because the resulting benefits accrue to others. Purely altruistic acts are likely rare, and motivation for most human activities is not accurately described as either entirely altruistic or egoistic, but having elements of each. Accurately assessing motives of research participants can be very challenging, so benefits accruing from altruistic motives cannot be assumed and best practices would not make them a consideration in EPA CHIE trials.

- *Financial benefits*: Payment of healthy volunteers for research is usually considered apart from the risk–benefit calculus by IRBs. It is discussed here because payment is often necessary to secure recruitment of healthy volunteers, and because many subjects clearly see money as an associated benefit, perhaps as the most important benefit, for participating. Payments are typically offered to compensate for the time required by participation, lost wages, travel costs, and inconvenience and other obligations imposed by the study (NBAC 2001, NRC, 2004a). The ideal amount of compensation is a combination of fair payment for the time and other burdens required, and a middle ground between enough money to attract potential subjects, but not so much as to create an undue influence. Payments or other inducements are "undue" when they tempt volunteers to assume risks they would not otherwise take, or "prompt subjects to lie or conceal information that would otherwise disqualify them as participants" (Fisher, 2009; Macklin, 1981; Miller, 2003). Some have suggested that a minimum hourly wage for research participation with no upper limits on the compensation is an appropriate standard (Shamoo and Resnik, 2006). In CHIE studies the appropriate level of compensation, including consideration of upper limits, is best determined for each trial by common agreement of investigators and the IRB.

Often both benefit and risk are portrayed as the same for all research participants, but both are better understood as mediated by social position and financial need (Fisher, 2015). Care needs to be taken when recruiting low-income volunteers, students, others in vulnerable social or financial positions, and potentially study repeaters (three of the EPA studies reviewed included repeat participants) for whom monetary rewards could alter their judgments about risks.

RESEARCHER COMMUNICATION AND PARTICIPANT UNDERSTANDING OF INFORMED CONSENT

Risk Perception and Risk Communication

Risk perception is a subjective assessment by a person based on his or her beliefs regarding the probability of a potential hazardous event or activity and how it will affect him or her. Individual philosophies, principles, and past experiences can shape one's beliefs about perceived risk. The severity of the risk and the overall public opinion of the risk can also affect individual risk perceptions (Beecher et al., 2005; Slovic, 1987). All of those factors need to be considered in the development of informed-consent documentation and in the communication by investigators about human-subjects research (Raich et al., 2001). A person's recall or ability to relate to an experience with a particular risk can help improve risk comprehension (Anderson and Iltis, 2008; Keller, 2011).

Risk communication is "any purposeful exchange of information about health or environmental risks between interested parties" (Covello et al., 1987). This information incorporates understanding, ideas, and actions as they relate to risks (Anderson and Iltis, 2008; NRC, 1989, 2005). In the communication of risk associated with exposure to putative hazards, comparisons may be made to help research participants understand the risks they might encounter from exposures in the study compared to risks they might encounter from exposures in their daily lives (see Chapter 6).

There are several ways to communicate the risk of an exposure, and assessing the most effective strategies for comparing risk of one exposure to other similar exposures is an important field of study (Johnson, 2004a, 2004b; Keller, 2011). The effectiveness of comparisons might depend on the target audience's risk perceptions and their cognitive processes used to assess the risk and on the specific exposure to the risk (Williams, 2004). Exposure comparison can be used to help individuals understand a risk and its potential effects by drawing from their past experiences with exposures to other hazards in order to make the comparison more meaningful. When utilizing exposure comparison as a communication strategy, it is essential to compare exposures related to similar kinds of risk (CDC, 2014). Utilizing more familiar and comparable risks can be beneficial, especially for those with less experience with risk interpretation. For example, the risk of lung cancer or respiratory disease associated with cigarette smoking is

considered common knowledge; therefore, utilizing this exposure as a comparison for gauging other hazards thought to be linked to lung cancer, cardiovascular diseases, or respiratory diseases can result in more accurate interpretations of risk levels even among individuals with poorer numeracy skills (Keller, 2011; Keller et al., 2009). Comparing exposures related to risks that are less similar in nature (e.g., likelihood of dying from smoking versus from an industrial accident) can be confusing and misleading (Sandman, 1987). As per Chapter 6, it is recommended that, for communication with study participants, short-term risks be characterized in a descriptive, comparative manner using exposure equivalents that are relevant and understandable for participants. Also, comparative exposure scenarios should be fully documented so that the reasonableness of the comparison can be evaluated.

Participant Comprehension of Informed Consent

People's perceptions and comprehension of risk on informed-consent forms and throughout the informed consent process are often influenced by the presentation and framing of the information and literacy levels (Anderson and Iltis, 2008; Fortun et al., 2008; Keller and Siegrist, 2009; Peters et al., 2011; Raich et al., 2001; Reynolds and Nelson, 2007; Sand et al., 2012; Stunkel et al., 2010). Language, length of forms, and the presentation of the material, such as use of graphics and categorization of information, influence understanding of risk (Raich et al., 2001). In addition, information framed positively produces a different level of risk perception compared with negatively framed information. An example of this is using a phrase such as "chance of survival" as opposed to "chance of death," which could influence participants' perceptions about an exposure and, therefore, whether or not they choose to participate (Anderson and Iltis, 2008; Peters et al., 2011).

One's understanding of basic mathematical concepts can influence risk interpretation. Health professionals tend to overestimate patients' health literacy skills and the clarity of their own communication (Dickens et al., 2013; Howard et al., 2013). Results from the 2012 Program for the International Assessment of Adult Competencies, for example, demonstrated that the U.S. average literacy score was 270 or at Level 2 (Level 5 or scores between 376 and 500 demonstrate highest proficiency) (U.S. Department of Education, 2012). Health care professionals and researchers need to be aware of a patient's literacy level, especially concerning complex medical issues. A patient might struggle with unfamiliar terminology, which can impair his or her ability to receive needed care, which exacerbates the disparity among individuals with lower education levels (Davis et al., 2002). This is relevant not only in discussions between patient and provider, but also in the development of written materials, such as research and medical consent forms. Studies examining the reading level of consent forms find that consent resources are written at an upper high school (grades 10-12) to college (grade 13) level, which exceeds the average literacy level of general populations (Ittenbach et al., 2015; Paasche-Orlow et al., 2003, 2013; Sand et al., 2012; Taylor and Bramley, 2012). Sample consent language from one of the CHIE studies documents provided to the committee by EPA (OMEGACON) includes the following, which is written at a college reading level: "This gene, glutathione-S-transferase ($GSTM_1$) is one of several genes responsible for protecting your body against oxidants such as some air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants."

Assessing Participant Comprehension

There is an important need to ensure individuals' comprehension of informed-consent documentation and messages communicated verbally by study researchers. Much of the work conducted in this area has been focused on consent for participation in clinical trials (IOM, 2015).

A review of 44 intervention studies using strategies for improving comprehension of informed consent demonstrated that interventions providing additional written information (such as information specific to a procedure or brief information booklet in addition to the written consent form), audio/visual (AV) methods (such as mainly use of AV materials in addition to standard informed consent), extended discus-

sions (such as discussions about procedural risks or more broadly about the procedure itself), and using test-feedback methods (such as asking patients to repeat back information from consent discussions) were considered effective in improving comprehension about risks and general knowledge about a medical procedure (Schenker et al., 2010). Most studies, however, only assessed understanding of procedural risk, and not about benefits, alternatives, and general knowledge of the research. Only 6 of the 44 studies reviewed assessed all four of these components of comprehension, although 32 studies found that methods applied were effective in improving comprehension of risk and/or general knowledge. An earlier systematic review of interventions aimed to increase understanding of the consent process in clinical research suggested that multimedia and enhanced discussions about consent forms have limited effects on improving participants' comprehension of research protocols and that one-on-one discussions with research staff might have a stronger influence on understanding (Flory and Emanuel, 2004).

Other research, however, has demonstrated that presenting risk information in a multimedia/video (nonprinted) format alone, or to supplement printed forms, also has resulted in improved comprehension of health and risk information (Wang et al., 2015; Wanzer et al., 2010), including improved understanding of research consent-related information by individuals with lower literacy (Afolabi et al., 2015). Utilizing multimedia formats to supplement presentation of the informed-consent form resulted in greater comprehension among parents and guardians regarding a child's endoscopy procedure (Wanzer et al., 2010). An EPA training course on the consent process recommends oral presentations, educational materials (such as printed brochures), and videos to provide participants with additional information about study procedures (EPA 2014c).

Assessing Participant Comprehension in EPA CHIE Studies

For the CHIE studies considered by the committee, EPA used a consent checklist with 13 statements to gauge participant comprehension. Participants are asked to respond "yes" or "no" to statements, including the following:

- "I understand that I will undergo controlled exposure to the air pollutant ozone (at a concentration of 300 ppb) during the course of this study";
- "I understand that there are risks associated with my participation in the study"; and
- "A study team member discussed potential risks associated with participation in this study, and the measures that will be taken by the study team to reduce risk, with me."

After participants respond to the checklist items, they are given the opportunity to ask questions about the study and their involvement in it.

Additional tools and approaches for ensuring participants' understanding of research protocols that could improve EPA's checklist method are described here.

Ensuring participants have answers to key questions: The Institute of Medicine workshop and report on informed consent and health literacy included presentation of a curriculum for community members making decisions regarding research participation (IOM, 2015). A list of 10 key questions that individuals should ask of a researcher prior to participating in a study was presented (see IOM workgroup report, Chapter 3). These questions are applicable to EPA's CHIE studies. Questions were as follows: (1) What is the main purpose of the study?; (2) What will I be asked to do during the study?; (3) How will I benefit from participating in this study?; (4) What are the possible risks?; (5) How will the results be shared?; (6) How will my personal information be kept confidential?; (7) How long is the study going to last?; (8) Are there any reimbursements or incentives offered?; (9) Who is funding the study?; and (10) What are the credentials of the researcher and the researcher's institution?

Brief screening tool: The Single Item Literacy Screener (SILS) is a published tool designed to quickly assess comprehension. The SILS uses a single question to determine a participant's ability to read and comprehend health-related information. An individual would be posed with the question, *"How often do you need to have someone help you when you read instructions, pamphlets, or other written material from your*

doctor or pharmacy?" Responses are measured with a Likert-type scale ranging from never needing help to always needing help. Individuals scoring at or below a determined level should be provided with additional resources to facilitate comprehension of the materials (Morris et al., 2006). While this is a quick and easy literacy assessment, it will not be relevant for individuals who may not ever read such materials and an analogous screening question may need to be developed for this group of individuals.

Study-specific survey instrument: Studies have gauged participants' comprehension of clinical research study protocols (Chappuy et al., 2010; Ittenbach et al., 2015; Stunkel et al., 2010). Few published tools exist that ask specific questions about the consent form language itself in order to gauge comprehension of the specific aspects the study and the type of participation involved. Shafiq and Malhotra (2011) developed a 24-item survey to evaluate clinical research participants' understanding of an informed-consent form. The questionnaire was developed specifically for the study being conducted. Multiple-choice response options were provided to participants regarding topics such as study background (nine questions), study design (six questions), and participants' rights (nine questions). By having potential participants complete their survey, they were able not only to evaluate people's comprehension of the information, but also to help build trust between researchers and individuals participating in the study.

Teach-back methods: More limited research has been conducted on verbal health literacy or people's understanding of prose or numeric information received through oral exchanges (Nouri and Rudd, 2015). One important strategy being considered is referred to as "teach-to-goal" or the "teach-back" method. The method involves one-on-one discussions between the study team member and a participant. This method not only helps the participant comprehend the information, it also allows the study team to assess the participant's level of understanding (Tamariz et al., 2013). Teach-back involves a three-part process: (1) assessing comprehension, (2) offering feedback, and (3) reevaluating comprehension. The goal of this process is to provide a comprehensive explanation of the interventions to which participants will be subjected, tailored to their level of understanding. This method also provides an opportunity for the study team to determine whether the information provided was understood by the participants (Flowers, 2006; Kripalani et al., 2008; Tamura-Lis, 2013). Using teach-to-goal methodology in the review of the research consent process has demonstrated significant improvements in comprehension by individuals from diverse backgrounds and with lower literacy levels (Kripalani et al., 2008; Sudore et al., 2006). Examples of teach-back educational materials include the following:

- Agency for Healthcare Research and Quality (AHRQ) Reference Guide on teach-back methods, http://www.ahrq.gov/sites/default/files/wysiwyg/professionals/education/curriculum-tools/shareddecisionmaking/tools/tool-6/share-tool6.pdf;
- AHRQ's Teach-Back Self-Evaluation and Tracking Log, http://www.ahrq.gov/sites/default/files/wysiwyg/professionals/quality-patient-safety/quality-resources/tools/literacy-toolkit/healthliteracytoolkit.pdf; and
- University of North Carolina at Chapel Hill Sample teach-back method videos, http://www.nchealthliteracy.org/teachingaids.html.

RECOMMENDATIONS

Informed-Consent Document Development

Overall, there are some limitations with current consent documents for the CHIE studies. Some of the existing informed-consent documents reviewed by the committee contain complicated and technical language that requires high literacy and numeracy skills. In addition, exposure comparators, presented in those documents, might not be familiar or relevant to participants (see Chapter 6). **EPA should use a more plain-language presentation of risk information in consent documents for all IRB protocols. In general, development of consent documents should include these steps:**

- **Use a credible evidence standard (such as empirical research, experience, or scientific or mathematical principles) to determine what risks can reasonably be considered foreseeable in the study.**
- **Explore exposure-related health effects that have been reported in studies that used similar CHIE study interventions and in epidemiologic and animal studies.**
- **Consider how content should be framed within the document (such as positive frame versus negative frame) and the influence this might have on potential participants. EPA should conduct a pilot test of consent information that uses different frames and assess which has more of an impact on participants consenting to participate in CHIE studies.**
- **Provide accumulated information on the occurrence of serious adverse events associated with previous CHIE studies, and the resolution of these events (as discussed in Chapter 5) to illustrate that a study involves risks of serious adverse events that can be anticipated and those that cannot be anticipated.**
- **Characterize reasonably foreseeable risks by using an easily understood perspective and incorporating relevant exposure comparator scenarios into language about the study (see Chapter 6). The comparators should be evidence based and their development explained.**
- **Include and delineate all reasonably foreseeable risks and any risks likely to be perceived as important by the participants.** CHIE studies typically impart a very small increase in the cumulative exposure to ambient air pollution over an individual's lifetime, and there is no credible evidence to suggest that chronic effects be considered among the reasonably foreseeable risks of those studies. However, potential participants might be concerned about long-term risks because of associations between long-term exposure to air pollution and chronic effects. Therefore the likelihood of chronic effects needs to be included in informed-consent communications.
- **Describe uniformly the risks from experimental procedures that are used often (such as bronchoscopy) and indicate how the risk profile of the study subjects (such as mild asthmatic) has been taken into account.**
- **Communicate clearly that CHIE studies offer no medical benefit to exposed individuals.**
- **Conduct readability assessments using validated instruments such as SMOG or Flesch-Kincaid Reading Grade Level on all content intended for study participants to ensure grade 6-8 level. See McLaughlin (1969) and Kincaid et al. (1975).**
- **Supplement consent documents with audiovisual materials to present consent information to ensure that study information is more accessible for individuals with lower literacy and numeracy, and that the language is culturally appropriate.**

Assessing Participant Comprehension and Consent Communication

EPA should strive in all CHIE studies to ensure that obtaining informed consent is a two-way discussion with potential participants. EPA is currently employing a 13-statement checklist to assess participants' understanding of informed-consent documents. **To improve the way it ensures participants' understanding of research protocols, EPA should modify the current informed-consent checklist to involve a more in-depth assessment of participant comprehension of risks of participation and societal benefits accrued by these studies. The agency should use tools and approaches described in this chapter, including the following:**

- **Asking participants specific questions about the study to ensure understanding of consent form,**
- **Using teach-back methods with participants during the consent process, and**
- **Continuing to provide participants with the opportunity to ask questions.**

References

Afolabi, M.O., N. McGrath, U. D'Alessandro, B. Kampmann, E.B. Imoukhuede, R.M. Ravinetto, N. Alexander, H.J. Larson, D. Chandramohan, and K. Bojang. 2015. A multimedia consent tool for research participants in the Gambia: A randomized controlled trial. Bull. World Health Org. 93(5):320-328A.

Agarwal, S.K., G. Heiss, R.G. Barr, P.P. Chang, L.R. Loehr, L.E. Chambless, E. Shahar, D.W. Kitzman, and W.D. Rosamond. 2012. Airflow obstruction, lung function, and risk of incident heart failure: The Atherosclerosis Risk in Communities (ARIC) study. Eur. J. Heart Fail. 14(4):414-422.

Alexis, N., B. Urch, S. Tarlo, P. Corey, D. Pengelly, P. O'Byrne, and F. Silverman. 2000. Cyclooxygenase metabolites play a different role in ozone-induced pulmonary function decline in asthmatics compared to normals. Inhal. Toxicol. 12(12):1205-1224.

Alexis, N.E., J.C. Lay, M. Hazucha, B. Harris, M.L. Hernandez, P.A. Bromberg, H. Kehrl, D. Diaz-Sanchez, C. Kim, R.B. Devlin, and D.B. Peden. 2010. Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. Inhal. Toxicol. 22(7):593-600.

Amdur, M.O., W.W. Melvin, Jr., P. Drinker. 1953. Effects of inhalation of sulphur dioxide by man. Lancet 262(678():758-759.

Anderson, E.E., and A.S. Iltis. 2008. Assessing and improving research participants' understanding of risk: Potential lessons from the literature on physician-patient risk communication. J. Empir. Res. Hum. Res. Ethics 3(3):27-37.

Attfield, M., P. Schleiff, J. Lubin, A. Blair, P. Stewart, R. Vermeulen, J. Cobble, and D. Silverman. 2012. Effects of diesel exhaust among non-metal miners: A cohort mortality study with emphasis on lung cancer. J. Natl. Cancer Inst. 104(11):869-883.

Barregard, L., G. Sallsten, P. Gustafson, L. Andersson, L. Johansson, S. Basu, and L. Stigendal. 2006. Experimental exposure to wood-smoke particles in health humans: Effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal. Toxicol. 18(11):845-853.

Barregard, L., G. Sallsten, L. Andersson, A.C. Almstrand, P. Gustafson, M. Andersson, and A.C. Olin. 2008. Experimental exposure to wood smoke: Effects on airway inflammation and oxidative stress. Occup. Environ. Med. 65(5):319-324.

Basu, R. 2009. High ambient temperature and mortality: A review of epidemiologic studies from 2001 to 2008. Environ. Health 8:40.

Basu, R., and B. Malig. 2011. High ambient temperature and mortality in California: Exploring the roles of age, disease, and mortality displacement. Environ. Res. 111(8):1286-1292.

Beckett, W.S., D.F. Chalupa, A. Pauly-Brown, D.M. Speers, J.C. Stewart, M.W. Frampton, M.J. Utell, L.S. Huang, C. Cox, W. Zareba, and G. Oberdörster. 2005. Comparing inhaled ultrafine versus fine zinc oxide particles in healthy adults: A human inhalation study. Am. J. Respir. Crit. Care. Med. 171(10):1129-1135.

Beecher, N., E. Harrison, N. Goldstein, M. mcDaniel, P. Field, and L. Susskind. 2005. Risk perception, risk communication, and stakeholder involment for biosolids management and research. J. Environ. Qual. 34(1):122-128.

Behndig, A.F., I.S. Mudway, J.L. Brown, N. Stenfors, R. Helleday, S.T. Duggan, S.J. Wilson, C. Boman, F.R. Cassee, A.J. Frew, F.J. Kelly, T. Sandstrom, and A. Blomberg. 2006. Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur. Respir. J. 27(2):359-365.

Bell, M.L., A. McDermott, S.L. Zeger, J.M. Samet, and F. Dominici. 2004. Ozone and short-term mortality in 95 US urban communities, 1987–2000. JAMA 292(19):2372-2378.

Bell, M.L., K. Ebisu, R.D. Peng, J.M. Samet, and F. Dominici. 2009. Hospital admissions and chemical composition of fine particle pollution. Am. J. Respir. Crit. Care Med. 179(12):1115-1120.

Blanchard, C.L., G.M. Hidy, S. Tannenbaum, E.S. Edgerton, and B.E. Hartsell. 2013. The Southeastern Aerosol Research and Characterization (SEARCH) study: Temporal trends in gas and PM concentrations and composition, 1999-2010. J. Air Waste Manage. Assoc. 63(3):247-259.

Blomberg, A., H. Törnqvist, L. Desmyter, V. Deneys, and C. Hermans. 2005. Exposure to diesel exhaust nanoparticles does not induce blood hypercoagulability in an at-risk population. J. Thromb. Haemost. 3(9):2103-2105.

Boehm Vock, L.F., B.J. Reich, M. Fuentes, and F. Dominici. 2015. Spatial variable selection methods for investigating acute health effects of fine particulate matter components. Biometrics 71(1):167-177.

Bräuner, E.V., P. Møller, L. Barregard, L.O. Dragsted, M. Glasius, P. Wåhlin, P. Vinzents, O. Raaschou-Nielsen, and S. Loft. 2008. Exposure to ambient concentrations of particulate air pollution does not influence vascular function or inflammatory pathways in young healthy individuals. Part. Fibre Toxicol. 5:13.

Brook, R.D., J.R. Brook, B. Urch, R. Vincent, S. Rajagopalan, and F. Silverman. 2002. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105(13):1534-1536.

Brook, R.D., B. Franklin, W. Cascio, Y. Hong, G. Howard, M. Lipsett, R. Luepker, M. Mittleman, J. Samet, S.C. Smith, and I. Tager. 2004. Air pollution and cardiovascular disease: A statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109(21):2655-2671.

Brook, R.D., B. Urch, J.T. Dvonch, R.L. Bard, M. Speck, G. Keeler, M. Morishita, F.J. Marsik, A.S. Kamal, N. Kaciroti, J. Harkema, P. Corey, F. Silverman, D.R. Gold, G. Wellenius, M.A. Mittleman, S. Rajagopalan, and J.R. Brook. 2009. Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. Hypertension 54(3):659-667.

Brook, R.D., S. Rajagopalan, C.A. Pope, III, J.R. Brook, A. Bhatnagar, A.V. Diez-Roux, F. Holguin, Y. Hong, R.V. Luepker, M.A. Mittleman, A. Peters, D. Siscovick, S.C. Smith, Jr., L. Whitsel, and J.D. Kaufman. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 121(21):2331-2378.

Broun, P. 2012. Request to Review Recent Human Research Studies Involving Concentrated Airborne Particles Conducted by the EPA and UNC. Letter from Rep. Paul Broun, Chairman, Subcommittee on Investigations and Oversight, U.S. House of Representatives Committee on Science, Space, and Technology to Arthur A. Elkins, Jr., Inspector General, U.S. Environmental Protection Agency, Washington DC. October 18, 2012 [online]. Available: https://science.house.gov/sites/republicans.science.house.gov/files/documents/2012%2010%2018%20Broun%20to%20Elkins%20re%20PM2.5.pdf [accessed May 24, 2016].

Brown, J.S., J.A. Graham, L.C. Chen, E.M. Postlethwait, A.J. Ghio, W.M. Foster, and T. Gordon. 2007. Panel discussion review: Session four—assessing biological plausibility of epidemiological findings in air pollution research. J. Exposure Sci. Environ. Epidemiol. 17(Suppl. 2):S97-S105.

Buzzard, N., N.N. Clark, and S.E. Guffey. 2009. Investigation into pedestrian exposure to near-vehicle exhaust emissions. Environ. Health 8:13.

Carlsten, C., D.J. Kaufman, A. Peretz; C.A. Trenga, L. Sheppard, and J.H. Sullivan. 2007. Coagulation markers in healthy human subjects exposed to diesel exhaust. Thromb. Res. 120(6):849-855.

CDC (Centers for Disease Control and Prevention). 2014. Crisis and Emergency Risk Communication [online]. Available: https://emergency.cdc.gov/cerc/resources/pdf/cerc_2014edition.pdf [accessed March 2, 2017].

Chappuy, H., A. Baruchel, G. Leverger, C. Oudot, B. Brethon, S. Haouy, A. Auvrignon, D. Davous, F. Doz, and J.M. Tréluyer. 2010. Parental comprehension and satisfaction in informed consent in paediatric clinical trials: A prospective study on childhood leukaemia. Arch. Dis. Child 95(10):800-804.

Chou, R. 2014. In the clinic. Low back pain. Ann. Intern. Med. 160(11):ITC6-1.

Coble, J.B., P.A. Stewart, R. Vermeulen, D. Yereb, R. Stanevich, A. Blair, D.T. Silverman, and M. Attfield. 2010. The diesel exhaust in miners study: II. Exposure monitoring surveys and development of exposure groups. Ann. Occup. Hyg. 54(7):747-761.

Correia, A.W., C.A. Pope, III, D.W. Dockery, Y. Wang, M. Ezzati, and F. Dominici. 2013. Effect of air pollution control on life expectancy in the United States: An analysis of 545 U.S. counties for the period from 2000 to 2007. Epidemiology 24(1):23-31.

Covello, V.T., D. von Winterfeldt, and P. Slovic. 1987. Communicating scientific information about health and environmental risks: Problems and opportunities from a social and behavioral perspective. Pp. 221-239 in Uncertainty in Risk Assessment, Risk Management, and Decision Making, V.T. Covello, L.B. Lave, A. Moghissi, and V.R.R. Uppuluri, eds. New York: Plenum Press.

Cox, L.A., Jr., and D.A. Popken. 2015. Has reducing fine particulate matter and ozone caused reduced mortality rates in the United States? Ann. Epidemiol. 25(3):162-173.

Crouse, D.L., P.A. Peters, A. van Donkelaar, M.S. Goldberg, P.J. Villeneuve, O. Brion, S. Khan, D.O. Atari, M. Jerrett, C.A. Pope, III, M. Brauer, J.R. Brook, R.V. Maetin, D. Stieb, and R.T. Burnett. 2012. Risk of nonaccidental and cardiovascular mortality in relation to long-term exposure to low concentrations of fine particulate matter: A Canadian national-level cohort study. Environ. Health Perspect. 120(5):708-714.

Crump, K.S., C. Van Landingham, S.H. Moolgavkar, and R. McClellan. 2015. Reanalysis of the DEMS nested case-control study of lung cancer and diesel exhaust: Suitability for quantitative risk analysis. Risk Anal. 35(4):676-700.

Crump, K.S., C.V. Landingham, and R.O. McClellan. 2016. Influence of alternative exposure estimates in DEMS miners study: Diesel exhaust and lung cancer. Risk Anal. 36(9):1803-1812.

Davis, T.C., M.V. Williams, E. Marin, R.M. Parker, and J. Glass. 2002. Health literacy and cancer communication. Ca Cancer J. Clin. 52(3):134-149.

Devlin, R.B., W.F. Mcdonnell, R. Mann, S. Becker, D.E. House, D. Schreinemachers, and H.S. Koren. 1991. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am. J. Respir. Cell Mol. Biol. 4(1):72-81.

Devlin, R.B., L.J. Folinsbee, F. Biscardi, G. Hatch, S. Becker, M.C. Madden, M. Robbins, and H.S. Koren. 1997. Inflammation and cell damage induced by repeated exposure of humans to ozone. Inhal. Toxicol. 9(3):211-235.

Devlin, R.B., A.J. Ghio, H. Kehrl, G. Sanders, and W. Cascio. 2003. Elderly humans exposed to concentrated air pollution particles have decreased heart rate variability. Eur. Respir. J. Suppl. 40:76s-80s.

Devlin, R.B., K.E. Duncan, M. Jardim, M.T. Schmitt, A.G. Rappold, and D. Diaz-Sanchez. 2012. Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126(1):104-111.

Devlin, R.B., C.B. Smith, M.T. Schmitt, A.G. Rappold, A. Hinderliter, D. Graff, and M.S. Carraway. 2014. Controlled exposure of humans with metabolic syndrome to concentrated ultrafine ambient particulate matter causes cardiovascular effects. Toxicol. Sci. 140(1):61-72.

Dickens, C., B.L. Lambert, T. Cromwell, and M.R. Piano. 2013. Nurse overestimation of patients' health literacy. J. Health Commun. 18(Suppl. 1):62-69.

Dockery, D.W, and J.H. Ware. 2015. Cleaner air, bigger lungs. N. Engl. J. Med. 372(10):970-972.

Dominici, F., Y. Wang, A.W. Correia, M. Ezzati, C.A. Pope, III, and D.W. Dockery. 2015. Chemical composition of fine particulate matter and life expectancy: In 95 U.S. counties between 2002 and 2007. Epidemiology 26(4):556-564.

Emanuel, E.J., D. Wendler, and C. Grady. 2000. What makes clinical research ethical? JAMA 283(20):2701-2711.

Enstrom, J.E. 2005. Fine particulate air pollution and total mortality among elderly Californians, 1973-2002. Inhal. Toxicol. 17(14):803-816.

EPA (U.S. Environmental Protection Agency). 2004. Air Quality Criteria for Particulate Matter, Vol. II. EPA/600/P-99/002bF. Office of Research and Development, EPA, Research Triangle Park, NC. October 2004.

EPA. 2006. Air Quality Criteria for Ozone and Related Photochemical Oxidants (Final Report). EPA/600/R-05/004aF-cF. National Center for Environmental Assessment, Office of Research and Development, EPA, Research Triangle Park, NC. February 2006.

EPA. 2009. Integrated Science Assessment for Particulate Matter. EPA/600/R-08/139F. National Center for Environmental Assessment, Office of Research and Development, EPA, Research Triangle Park. December 2009.

EPA. 2011. Policy Assessment for the Review of the Particulate Matter National Ambient Air Quality Standards. EPA/452/R-11-003. Office of Air Quality Planning and Standards, EPA, Research Triangle Park, NC. April 2011.

EPA. 2012. Provisional Assessment of Recent Studies on Health Effects of Particulate Matter Exposure EPA/600/R-12/056F. EPA, Washington, DC. December 2012.

EPA. 2013. Integrated Science Assessment of Ozone and Related Photochemical Oxidants. EPA/600/R-10/076F. National Center for Environmental Assessment, Office of Research and Development, EPA, Research Triangle Park, NC. February 2013.

EPA. 2014a. Improvements to EPA Policies and Guidance Could Enhance Protection of Human Study Subjects. Report No. 14-P-0154. Office of Inspector General. March 31, 2014 [online]. Available: https://www.epa.gov/sites/production/files/2015-09/documents/20140331-14-p-0154.pdf [accessed May 24, 2016].

EPA. 2014b. Policy Assessment of the Review of Ozone National Ambient Air Quality Standards. EPA-452/R-14-006. Office of Air and Radiation, Office of Air Quality Planning and Standards, EPA, Research Triangle Park, NC. August 2014.

EPA. 2014c. Human Subjects Research at the Environmental Protection Agency: Ethical Standards and Regulatory Requirements [online]. Available: https://www.epa.gov/sites/production/files/2014-12/documents/phre_training.pdf [accessed May 1, 2016].

EPA. 2015a. Preamble to the Integrated Science Assessments. EPA/600/R-15/067. National Center for Environmental Assessment, Office of Research and Development, EPA, Research Triangle Park, NC. November 2015 [online]. Available: https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244 [accessed June 8, 2016].

EPA. 2016a. Human Studies Review Board [online]. Available: https://www.epa.gov/osa/human-studies-review-board [accessed June 8, 2016].

EPA. 2016b. About the National Health and Environmental Effects Research Laboratory (NHEERL) [online]. Available: https://www.epa.gov/aboutepa/about-national-health-and-environmental-effects-research-laboratory-nheerl#why [accessed May 24, 2016].
EPA. 2016c. NAAQS Table. Criteria Air Pollutants [online]. Available: https://www.epa.gov/criteria-air-pollutants/naaqs-table [accessed May 25, 2016].
EPA. 2016d. Pollutants and Sources [online]. Available: https://www3.epa.gov/airtoxics/pollsour.html [accessed May 25, 2016].
EPA. 2016e. Integrated Science Assessment for Sulfur Oxides – Health Criteria (Second External Review Draft). EPA/600/R-16/351. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle park, NC. December 2016 [online]. Available: https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryID=326450 [accessed March 2, 2016].
EPA. 2016f. Particulate Matter ($PM_{2.5}$) Table [online]. Available: https://www.epa.gov/air-trends/particulate-matter-pm25-trends#pmnat [accessed on February 23, 2017].
EPA. 2017a. Basic Information about Human Subjects Research [online]. Available: https://www.epa.gov/osa/basic-information-about-human-subjects-research-0 [accessed February 27, 2017].
EPA. 2017b. Our Mission and What We Do [online]. Available: https://www.epa.gov/aboutepa/our-mission-and-what-we-do [accessed February 27, 2017].
EPA. 2017c. https://www.epa.gov/air-trends/particulate-matter-pm25-trends#pmnat (accessed on February 23, 2017)
Faden, R., T. Beauchamp, and N. King. 1986. A History and Theory of Informed Consent. New York: Oxford University Press.
FDA (Food and Drug Administration). 2009. Guidance for Industry Investigator Responsibilities—Protecting the Rights, Safety, and Welfare of Study Subjects. October 2009. Available: http://www.fda.gov/downloads/Drugs/.../Guidances/UCM187772.pdf [accessed May 24, 2016].
FDA. 2012. Guidance for Sponsors, Investigators, and Institutional Review Boards, Questions and Answers on Informed Consent Elements, 21 CFR § 50.25(c) [online]. Available: https://history.nih.gov/about/downloads/InformedConsentFinalRule-QAFeb2012-Final.pdf [accessed May 25, 2016].
Fisher, J. 2009. Medical Research for Hire: The Political Economy of Pharmaceutical Clinical Trials. New Brunswick, NJ: Rutgers University Press.
Fisher, J.A. 2015. Feeding and bleeding: The institutional banalization of risk to healthy volunteers in Phase I pharmaceutical clinical trials. Sci. Technol. Human Values 40(2):199-226.
Flory, J., and E. Emanuel. 2004. Interventions to improve research participants' understanding in informed consent for research: A systematic review. JAMA 292(13):1593-1601.
Flowers, L. 2006. Teach-back improves informed consent. OR Manager 22(3):25-26.
Folinsbee, L.J., J.F. Bedi, and S.M. Horvath. 1980. Respiratory responses in humans repeatedly exposed to low concentrations of ozone. Am. Rev. Respir. Dis. 121(3):431-439.
Folinsbee, L.J., W.F. McDonnell, and D.H. Horstman. 1988. Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. J. Air Waste Manage. Assoc. 38(1):28-35.
Folinsbee, L.J., D.H. Horstman, H.R. Kehrl, S. Harder, S. Abdul-Salaam, and P.J. Ives. 1994. Respiratory responses to repeated prolonged exposure to 0.12 ppm ozone. Am. J. Respir. Crit. Care Med. 149(1):98-105.
Fortun, P., J. West, L. Chalkley, A. Shonde, and C. Hawkey. 2008. Recall of informed consent information by healthy volunteers in clinical trials. QJM 101(8):625-629.
Frampton, M.W., J.C. Stewart, G. Oberdorster, P.E. Morrow, D. Chalupa, A.P. Pietropaoli, L.M. Frasier, D.M. Speers, C. Cox, L.S. Huang, and M.J. Utell. 2006. Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. Environ. Health Perspect. 114(1):51-58.
Frank, N.R., M.O. Amdur, J. Worcester, and J.L. Whittenberger. 1962. Effects of acute controlled exposure to SO_2 on respiratory mechanics in healthy male adults. J. Appl. Physiol. 17(2):252-258.
Gauderman, W.J, R. Urman, E. Avol, K. Berhane, R. McConnell, E. Rappaport, R. Chang, F. Lurmann, and F. Gilliland. 2015. Association of improved air quality with lung development in children. N. Engl. J. Med. 372(10):905-913.
Ghio, A.J., C. Kim, and R.B. Devlin. 2000. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. Am. J. Respir. Crit. Care Med. 162(3 Pt. 1):981-988.
Ghio, A.J., M. Bassett, T. Montilla, E.H. Chung, C.B. Smith, W.E. Cascio, and M.S. Carraway. 2012. Case report: Supraventricular arrhythmia after exposure to concentrated ambient air pollution particles. Environ. Health Perspect. 120(2):275-277.

Gold, D.R., and M.A. Mittleman. 2013. New insights into pollution and the cardiovascular system. Circulation 127(18):1903-1913.

Goldstein, I.F., K. Lieber, L.R. Andrews, F. Kazembe, G. Foutrakis, P. Huang, and C. Hayes. 1988. Acute respiratory effects of short-term exposures to nitrogen dioxide. Arch. Environ. Health 43(2):138-142.

Gong, H., Jr., W.S. Linn, S.L. Terrell, K.W. Clark, M.D. Geller, K.R. Anderson, W.E. Cascio, and C. Sioutas. 2004a. Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles. Inhal. Toxicol. 16(6-7):335-343.

Gong, H., Jr., W.S. Linn, S.L. Terrell, K.R. Anderson, K.W. Clark, C. Sioutas, W.E. Cascio, N. Alexis, and R.B. Devlin. 2004b. Exposures of elderly volunteers with and without chronic obstructive pulmonary disease (COPD) to concentrated ambient fine particulate pollution. Inhal. Toxicol. 16(11-12):731-744.

Gong, H., Jr., W.S. Linn, K.W. Clark, K.R. Anderson, M.D. Geller, and C. Sioutas. 2005. Respiratory responses to exposures with fine particulates and nitrogen dioxide in the elderly with and without COPD. Inhal. Toxicol. 17(3):123-132.

Gong, H., Jr., W.S. Linn, K.W. Clark, K.R. Anderson, C. Sioutas, N.E. Alexis, W.E. Cascio, and R.B. Devlin. 2008. Exposures of healthy and asthmatic volunteers to concentrated ambient ultrafine particles in Los Angeles. Inhal. Toxicol. 20(6):533-545.

Graff, D., W. Cascio, A. Rappold, H. Zhou, Y. Huang, and R. Devlin. 2009. Exposure to concentrated coarse air pollution particles causes mild cardiopulmonary effects in healthy young adults. Environ. Health Perspect. 117(7):1089-1094.

Greven, S., F. Dominici, and S.L. Zeger. 2011. In approach to the estimation of chronic air pollution effects using spatio-temporal information. J. Am. Stat. Assoc. 106(494):396-406.

Hamra. G., N. Guha. A Cohen, F. Laden, O. Raaschou-Nielsen, J. Samet, P. Vineis, F. Forastiere, P. Saldiva, T. Yorifuji, and D. Loomis. 2014. Outdoor particulate matter exposure and lung cancer: A systematic review and meta-analysis. Environ. Health Perspect. 122(9): 906-911.

Hand, J.L., B.A. Schichtel, W.C. Malm, and N.H. Frank. 2013. Spatial and temporal trends in $PM_{2.5}$ organic and elemental carbon across the United States. Adv. Meteorol. 2013:367674. doi: 10.1155/2013/367674.

Hao, Y., G. Zhang, B. Han, X. Xu, N. Feng, Y. Li, W. Wang, H. Kan, Z. Bai, Y. Zhu, W. Au, and Z. Xia. 2017. Prospective evaluation of respiratory health benefits from reduced exposure to airborne particulate matter. Int. J. Environ. Health Res. 27(2):126-135.

Hart, H.L.A., and T. Honore. 1985. Causation in the Law, 2nd Ed. Oxford: Clarendon Press.

Hazucha, M.J., and A.S. Lefohn. 2007. Nonlinearity in human health response to ozone: Experimental laboratory considerations. Atmos. Environ. 41(22):4559-4570.

Hazucha, M.J., D.V. Bates, and P.A. Bromberg. 1989. Mechanism of action of ozone on the human lung. J. Appl. Physiol. 67(4):1535-1541.

Hazucha, M.J., L.J. Folinsbee, and P.A. Bromberg. 2003. Distribution and reproducibility of spirometric response to ozone by gender and age. J. Appl. Physiol. 95(5):1917-1925.

Henderson, G.E., L.R. Churchill, A.M. Davis, M.M. Easter, C. Grady, S. Joffe, N. Kass, N.M.P. King, C.W. Lidz, F.G. Miller, D.K. Nelson, J. Peppercorn, B. Bluestone Rothschild, P. Sankar, B.S. Wilfond, and C.R. Zimmer. 2007. Clinical trials and medical care: Defining the therapeutic misconception. PLoS Med. 4(11):e324.

Hernandez, M.L., J.C. Lay, B. Harris, C.R. Esther, W.J. Brickey, P.A. Bromberg, D. Diaz-Sanchez, R.B. Devlin, S.R. Kleeberger, N.E. Alexis, and D.B. Peden. 2010. Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. J. Allergy Clin. Immunol. 126(3):537-544.

Hesterberg, T.W., C.M. Long, S.N. Sax, C.A. Lapin, R.O. McClellan, W.B. Bunn, and P.A. Valberg. 2011. Particulate matter in new technology diesel exhaust (NTDE) is quantitatively and qualitatively very different from that found in traditional diesel exhaust (TDE). J. Air Waste Manage. Assoc. 61(9):894-913.

Hill, A.B. 1965. The environment and disease: Association or causation? Proc. R. Soc. Med. 58:295-300.

Hill, G.N., and K.T. Hill. 2014. The People Law Dictionary: Foreseeable Risk. Fine Communication [online]. Available: http://dictionary.law.com/Default.aspx?selected=770 [accessed May 25, 2016].

Hoek, G., R.M. Krishnan, R. Beelen, A. Peters, B. Brunekreef, and J.D. Kaufman. 2013. Long-term air pollution exposure and cardiorespiratory mortality: A review. Environ. Health 12:43.

Howard, T., K.L. Jacobson, and S. Kripalani. 2013. Doctor talk: Physicians' use of clear verbal communication. J. Health Commun. 18(8):991-1001.

Hozawa, A, J.L. Billings, E. Shahar, T. Ohira, W.D. Rosamond, and A.R. Folsom. 2006. Lung function and ischemic stroke incidence: The Atherosclerosis Risk in Communities study. Chest 130(6):1642-1649.

Huang, Y.C., A.J. Ghio, J. Stonehuerner, J. McGee, J.D. Carter, S.C. Grambow, and R.B. Devlin. 2003. The role of soluble components in ambient fine particles-induced changes in human lungs and blood. Inhal. Toxicol. 15(4):327-342.

Hulzebos, E.H., H. Bomhof-Roordink, P.B. van de Weert-van Leeuwen, J.W. Twisk, H.G. Arets, C.K. van der Ent, and T. Takken. 2014. Prediction of mortality in adolescents with cystic fibrosis. Med. Sci. Sports Exerc. 46(11):2047-2052.

IOM (Institute of Medicine). 2014. Veterans and Agent Orange: Update 2012. Washington, DC: The National Academies Press.

IOM (Institute of Medicine). 2015. Informed Consent and Health Literacy: Workshop Summary. Roundtable on Health Literacy, Board on Population Health and Public Health Practice. Washington, DC: The National Academies Press.

Ito, K., S.F. De Leon, and M. Lippmann. 2005. Associations between ozone and daily mortality: Analysis and meta-analysis. Epidemiology 16(4):446-457.

Ittenbach, R.F., E.C. Senft, G. Huang, J.J. Corsmo, and J.E. Sieber. 2015. Readability and understanding of informed consent among participants with low incomes: A preliminary report. J. Empir. Res. Hum. Res. Ethics 10(5):444-448.

Johnson, B.B. 2004a. Risk comparisons, conflict, and risk acceptability claims. Risk Anal. 24(1):131-145.

Johnson, B.B. 2004b. Varying risk comparison elements: Effects on public reactions. Risk Anal. 24(1):103-114.

Josefson, D. 2001. Healthy woman dies in research experiment. BMJ 322(7302):1565.

Kahle, J.J., L.M. Neas, R.B. Devlin, M.W. Case, M.T. Schmitt, M.C. Madden, and D. Diaz-Sanchez. 2015. Interaction effects of temperature and ozone on lung function and markers of systemic inflammation, coagulation, and fibrinolysis: A crossover study of healthy young volunteers. Environ. Health Perspect. 123(4):310-316.

Katsouyanni, K., J.M. Samet, H.R. Anderson, R. Atkinson, A. Le Tertre, S. Medina, E. Samoli, G. Touloumi, R.T. Burnett, D. Krewski, T. Ramsay, F. Dominici, R.D. Peng, J. Schwartz, and A. Zanobetti. 2009. Air pollution and health: A European and North American Approach (APHENA). Res. Rep. Health Eff. Inst. 142:5-90.

Keller, C. 2011. Using a familiar risk comparison within a risk ladder to improve risk understanding by low numerates: A study of visual attention. Risk Anal. 31(7):1043-1054.

Keller, C., and M. Siegrist. 2009. Effect of risk communication formats on risk perception depending on numeracy. Med. Decis. Making 29(4):483-490.

Keller, C., M. Siegrist, and V. Visschers. 2009. Effect of risk ladder format on risk perception in high- and low-numerate individuals. Risk Anal. 29(9):1255-1264.

Kennedy, D. 2001. Death at Johns Hopkins. Science 293(5532):1013.

Kim, C.S., N.E. Alexis, A.G. Rappold, H. Kehrl, M.J. Hazucha, J.C. Lay, M.T. Schmitt, M. Case, R.B. Devlin, D.B. Peden, and D. Diaz-Sanchez. 2011. Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am. J. Respir. Crit. Care Med. 183(9):1215-1221.

Kincaid, J.P., R.P. Fishburne, R.L. Rogers, and B.S. Chissom. 1975. Derivation of New Readability Formula for Navy Enlisted Personnel. Navy Research Branch Report 8-75. [online]. Available: http://www.dtic.mil/dtic/tr/fulltext/u2/a006655.pdf [accessed March 2, 2017].

King, N. 2000. Defining and describing benefits appropriately in clinical trials. J. Law Med. Ethics 28(4): 332-343.

King, N. 2005. Glossary of basic ethical concepts in health care and research. Pp. 161-168 in The Social Medicine Reader, 2nd Ed., Vol. 1, N. King, G. Henderson, S. Estroff, J. Oberlander, and L. Churchill, eds. Durham, NC: Duke University Press.

Kioumourtzoglou, M.A., E. Austin, P. Koutrakis, F. Dominici, J. Schwartz, and A. Zanobetti. 2015. $PM_{2.5}$ and survival among older adults effect modification by particulate composition. Epidemiology 26(3):321-327.

Kleeberger, S.R. 1995. Genetic susceptibility to ozone exposure. Toxicol. Lett. 82/83:295-300.

Kleeberger, S.R., and D. Peden. 2005. Gene-environment interactions in asthma and other respiratory diseases. Annu. Rev. Med. 56:383-400.

Koren, H.S., R.B. Devlin, D.E. Graham, R. Mann, M.P. Mcgee, D.H. Horstman, W.J. Kozumbo, S. Becker, D.E. House, W.F. McDonnell, and P.A. Bromberg. 1989. Ozone-induced inflammation in the lower airways of human subjects. Am. J. Respir. Crit. Care Med. 139(2):407-415.

Kripalani, S., R. Bengtzen, L.E. Henderson, and T.A. Jacobson. 2008. Clinical research in low-literacy populations: Using teach-back to assess comprehension of informed consent and privacy information. IRB: Ethics Hum. Res. 30(2):13-19.

Laden, F., J. Schwartz, F.E. Speizer, and D.W. Dockery. 2006. Reduction in fine particulate air pollution and mortality: Extended follow-up of the Harvard Six Cities study. Am. J. Respir. Crit. Care Med. 173(6):667-672.

Lay, J.C., N.E. Alexis, S.R. Kleeberger, R.A. Roubey, B.D. Harris, P.A. Bromberg, M.J. Hazucha, R.B. Devlin, and D.B. Peden. 2007. Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals [letter]. J. Allergy Clin. Immunol. 120(3):719-722.

Leaderer, B.P., J.A. Stolwijk, R.T. Zagraniski, and M. Qing-Shan. 1984. Field study of indoor air contaminant level associated with unvented combustion sources. P. 84-33 in Proceedings of the77th Annual Meeting-Air Pollution Control Association, June 24-29, 1984, San Francisco, CA, Vol. 2. Pittsburgh, PA: Air Pollution Control Association.

Leaderer, B.P., R.T. Zagraniski, M. Berwick, and J.A. Stolwijk. 1986. Assessment of exposure to indoor air contaminants from combustion sources: Methodology and application. Am. J. Epidemiol. 124(2):275-289.

Lee, J.H., E.M. Song, Y.S. Sim, Y.J. Ryu, and J.H. Chang. 2011. Forced expiratory volume in one second as a prognostic factor in advanced non-small cell lung cancer. J. Thorac. Oncol. 6(2):305-309.

Lepeule, J., F. Laden, D. Dockery, and J. Schwartz. 2012. Chronic exposure to fine particle and mortality: An extended follow-up of the Harvard Six Cities study from 1974-2009. Environ. Health Perspect. 120(7):965-970.

Lippmann, M. 2014. Toxicological and epidemiological studies of cardiovascular effects of ambient air fine particulate matter ($PM_{2.5}$) and its chemical components: Coherence and public health implications. Crit. Rev. Toxicol. 39(10):865-913.

Lippmann, M., L.C. Chen, T. Gordon, K. Ito, and G.D. Thurston. 2013. National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components. Research Report No. 177. Boston: Health Effects Institute [online]. Available: http://pubs.healtheffects.org/getfile.php?u=934 [accessed May 27, 2016].

Lucking, A., M. Lundback, N. Mills, D. Faratian, S. Barath, J. Pourazar, F. Cassee, K. Donaldson, N. Boon, J. Badimon, T. Sandstorm, A. Blomberg, and D. Newby. 2008. Diesel exhaust inhalation increases thrombus formation in man. Eur. Heart J. 29(24):3043-3051.

Lund, A.K., J. Lucero, S. Lucas, M.C. Madden, J.D. McDonald, J.C. Seagrave, T.L. Knuckles, and M.J. Campen. 2009. Vehicular emissions induce vascular MMP-9 expression and activity associated with endothelin-1-mediated pathways. Arterioscler. Thromb. Vasc. Biol. 29(4):511-517.

Macklin, R. 1981. On paying money to research subjects: 'Due' and 'undue' inducements. IRB 3(5):1-6.

Madden, M.C., T. Stevens, M. Case, M. Schmitt, D. Diaz-Sanchez, M. Bassett, T.S. Montilla, J. Berntsen, and R.B. Devlin. 2014. Diesel exhaust modulates ozone-induced lung function decrements in healthy human volunteers. Part. Fibre Toxicol. 11:37.

McDonnell, W.F. 1996. Individual variability in human lung function responses to ozone exposure. Environ. Toxicol. Pharmacol. 2(2-3):171-175.

McDonnell, W.F., III, D.H. Horstman, S. Abdul-Salaam, and D.E. House. 1985. Reproducibility of individual responses to ozone exposure. Am. Rev. Respir. Dis. 131(1):36-40.

McDonnell, W.F., H.R. Kehrl, S. Abdul-Salaam, P.J. Ives, L.J. Folinsbee, R.B. Devlin, J.J. O'Neil, and D.H. Horstman. 1991. Respiratory response of humans exposed to low levels of ozone for 6.6 hours. Arch. Environ. Occup. Health 46(3):145-150.

McDonnell, W.F., P.W. Stewart, S. Andreoni, E. Seal, Jr., H.R. Kehrl, D.H. Horstman, L.J. Folinsbee, and M.V. Smith. 1997. Prediction of ozone-induced FEV1 changes: Effects of concentration, duration, and ventilation. Am. J. Respir. Crit. Care Med. 156(3):715-722.

McLaughlin, G.H. 1969. SMOG grading—A new readability formula. J. Reading 12(8):639-646.

Menezes, A.M., R. Perez-Padilla, F.C. Wehrmeister, M.V. Lopez-Varel, A. Muino, G. Valdivia, C. Lisboa, J.R. Jardim, M.M. de Oca, C. Talamo, R. Bielemann, M. Gazzotti, R. Laurenti, B. Celli, and C.G. Victora. 2014. FEV1 is a better predictor of mortality than FVC: The PLATINO Cohort Study. PLoS ONE 9(10):e109732.

Miller, F.G. 2003. Clinical research with healthy volunteers: An ethical framework. J. Investig. Med. 51(Suppl. 1):S2-S5.

Mills, N.L., H. Tornqvist, S.D. Robinson, M. Gonzalez, K. Darnley, W. MacNee, N.A. Boon, K. Donaldson, A. Blomberg, T. Sandstrom, and D.E. Newby. 2005. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112(25):3930-3936.

Mills, N.L., H. Tornqvist, M.C. Gonzalez, E. Vink, S.D. Robinson, S. Soderberg, N.A. Boon, K. Donaldson, T. Sandström, A. Blomberg, and D.E. Newby. 2007. Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. N. Engl. J. Med. 357(11):1075-1082.

Mills, N.L., S.D. Robinson, P.H. Fokkens, D.L. Leseman, M.R. Miller, D. Anderson, E.J. Freney, M.R. Heal, R.J. Donovan, A. Blomberg, T. Sandstrom, W. MacNee, N.A. Boon, K. Donaldson, D.E. Newby, and F.R. Cassee. 2008. Exposure to concentrated ambient particles does not affect vascular function in patients with coronary heart disease. Environ. Health Perspect. 116(6):709-715.

Moolgavkar, S.H., E.T. Chang, G. Luebeck, E.C. Lau, H. Watson, K. Crump and R.O. McClellan. 2015. Diesel engine exhaust and lung cancer mortality – time related factors in exposure and risk. Risk Anal. 35(4):663-675.

Morris, N.S., C.D. MacLean, L.D. Chew, and B. Littenberg. 2006. The Single Item Literacy Screener: Evaluation of a brief instrument to identify limited reading ability. BMC Family Practice 7(1):21.

Mozaffarian, D., E.J. Benjamin, A.S. Go, D.K. Arnett, M.J. Blaha, M. Cushman, S.R. Das, S. de Ferranti, J.P. Despres, H.J. Fullerton, V.J. Howard, M.D. Huffman, C.R. Isasi, M.C. Jimenez, S.E. Judd, B.M. Kissela, J.H. Lichtman, L.D. Lisabeth, S. Liu, R.H. Mackey, D.J. Magid, D.K. McGuire, E.R. Mohler, III, C.S. Moy, P. Muntner, M.E. Mussolino, K. Nasir, R.W. Neumar, G. Nichol, L. Palaniappan, D.K. Pandey, M.J. Reeves, C.J. Rodriguez, W. Rosamond, P.D. Sorlie, J. Stein, A. Towfighi, T.N. Turan, S.S. Virani, D. Woo, R.W. Yeh, M.B. Turner. 2016. Heart disease and stroke statistics—2016 update: A report from the American Heart Association. Circulation 133(4):e38-360.

Mudway, I.S., N. Stenfors, S.T. Duggan, H. Roxborough, H. Zielinski, S.L. Marklund, A. Blomberg, A.J. Frew, T. Sandstrom, and F.J. Kelly. 2004. An in vitro and in vivo investigation of the effects of diesel exhaust on human airway lining fluid antioxidants. Arch. Biochem. Biophys. 423(1):200-212.

NBAC (National Bioethics Advisory Commission). 2001. Ethical and Policy Issues in Research Involving Human Participants [online]. Available: https://bioethicsarchive.georgetown.edu/nbac/human/overvol1.html [accessed May 13, 2016].

NCPHSBBR (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research). 1979. The Belmont Report: Ethical Principles and Guidance for the Protection of Human Subjects of Research. Washington, DC: U.S. Government Printing Office [online]. Available: http://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/# [accessed May 16, 2016].

NIH (National Institutes of Health). 2010. What Is Cough? [online]. Available: https://www.nhlbi.nih.gov/health/health-topics/topics/cough [accessed May 25, 2016].

Nouri, S.S., and R.E. Rudd. 2015. Health literacy in the "oral exchange": An important element of patient–provider communication. Patient Educ. Couns. 98(5):565-571.

NRC (National Research Council). 1987. Biological markers in environmental health research. Environ. Health Perspect. 74:3-9 [online]. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474499/pdf/envhper00433-0008.pdf [accessed May 27, 2016].

NRC. 1989. Improving Risk Communication. Washington, DC: National Academy Press.

NRC. 2004a. Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues. Washington, DC: The National Academies Press.

NRC. 2004b. Air Quality Management in the United States. Washington, DC: The National Academies Press.

NRC. 2005. Communicating Toxicogenomics Information to Nonexperts: A Workshop Summary. Washington, DC: The National Academies Press.

NRC. 2006. Human Biomonitoring for Environmental Chemicals. Washington, DC: The National Academies Press.

NRC. 2007. Toxicity Testing in the 21st Century: A Vison and a Strategy. Washington, DC: The National Academies Press.

Nuernberg Military Tribunals. 1949. The proof as to war crimes and crimes against humanity. Permissible medical experiments. Pp. 181-182 in Trials of War Criminals before the Nuernberg Military Tribunals under Control Council Law No. 10, Vol. 2. Washington, DC: U.S. Government Printing Office [online]. Available: https://www.loc.gov/rr/frd/Military_Law/pdf/NT_war-criminals_Vol-II.pdf [accessed February 28, 2017].

OHRP (Office for Human Research Protections). 2007. Unanticipated Problems Involving Risks and Adverse Events Guidance [online]. Available: http://www.hhs.gov/ohrp/regulations-and-policy/guidance/reviewing-unanticipated-problems/ [accessed May 25, 2016].

OHRP (Office for Human Research Protections). 2014. Draft Guidance on Disclosing Reasonably Foreseeable Risks in Research Evaluating Standards of Care [online]. Available: http://www.hhs.gov/ohrp/regulations-and-policy/requests-for-comments/draft-guidance-disclosing-risk-in-standards-of-care/index.html [accessed May 25, 2016].

Paasche-Orlow, M.K., H.A. Taylor, and F.L. Brancati. 2003. Readability standards for informed-consent forms as compared with actual readability. N. Engl. J. Med. 348(8):721-726.

Paasche-Orlow, M.K., F.L. Brancati, H.A. Taylor, S. Jain, A. Pandit, and M.S. Wolf. 2013. Readability of consent form templates: A second look. IRB 35(4):12-19.

Peden, D.B., R.W. Setzer, Jr., and D.B. Devlin. 1995. Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. Am. J. Respir. Crit. Care Med. 151(5):1336-1345.

Peden, D.B., B. Boehlecke, D. Horstman, and R. Devlin. 1997. Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. J. Allergy Clin. Immunol. 100(6 Pt. 1):802-808.

Peretz, A., E.C. Peck, T.K. Bammler, R.P. Beyer, J.H. Sullivan, C.A. Trenga, S. Srinouanprachnah, F.M. Farin, and J.D. Kaufman. 2007. Diesel exhaust inhalation and assessment of peripheral blood mononuclear cell gene transcription effects: An exploratory study of healthy human volunteers. Inhal. Toxicol. 19(14):1107-1119.

Peretz, A., J.H. Sullivan, D.F. Leotta, C.A. Trenga, F.N. Sands, J. Allen, C. Carlsten, C.W. Wilkinson, E.A. Gill, and J.D. Kaufman. 2008. Diesel exhaust inhalation elicits acute vasoconstriction in vivo. Environ. Health Perspect. 116(7):937-942.

Peters, E., P.S. Hart, and L. Fraenkel. 2011. Informing patients the influence of numeracy, framing, and format of side effect information on risk perceptions. Med. Decis. Making 31(3):432-436.

Pietropaoli, A.P., M.W. Frampton, R.W. Hyde, P.E. Morrow, G. Oberdorster, C. Cox, D.M. Speers, L.M. Frasier, D.C. Chalupa, L.S. Huang, and M.J. Utell. 2004. Pulmonary function, diffusing capacity, and inflammation in healthy and asthmatic subjects exposed to ultrafine particles. Inhal. Toxicol. 16(Suppl. 1):59-72.

Pleasant, A., M. Rooney, C. O'Leary, L. Myers, and R. Rudd. 2016. Strategies to Enhance Numeracy Skills. Discussion Paper, May 5 [online]. Available: https://nam.edu/wp-content/uploads/2016/05/Strategies-to-Enhance-Numeracy-Skills.pdf [accessed March 2, 2017].

Pope, C.A., III, M. Ezzati, and D.W. Dockery. 2009. Fine-particulate air pollution and life expectancy in the United States. N. Engl. J. Med. 360(4):376-386.

Pope, C.A., III, M. Ezzati, and D.W. Dockery. 2013. Fine particulate air pollution and life expectancies in the United States: The role of influential observations. J. Air Waste Manage. Assoc. 63(2):129-132.

Raich, P.C., K.D. Plomer, and C.A. Coyne. 2001. Literacy, comprehension, and informed consent in clinical research. Cancer Invest. 19(4):437-445.

Resnik, D.B. 2012. Limits on risks for healthy volunteers in biomedical research. Theor. Med. Bioeth. 33(2):137-149.

Resnik, D.B. 2013. What are reasonably foreseeable risks? Am. J. Bioeth. 13(12):29-30.

Reynolds, W.W., and R.M. Nelson. 2007. Risk perception and decision processes underlying informed consent to research participation. Soc. Sci. Med. 65(10):2105-2115.

Rid, A., and D. Wendler. 2011. A framework for risk-benefit evaluations in biomedical research. Kennedy Inst. Ethics J. 21(2):141-179.

Rid, A., E.J. Emanuel, and D. Wendler. 2010. Evaluating the risks of clinical research. JAMA 304(13):1472-1479.

Rosenthal, E. 1996. New York Seeks to Tighten Rules on Medical Research. New York Times, September 27, 1996 [online]. Available;
http://www.nytimes.com/1996/09/27/nyregion/new-york-seeks-to-tighten-rules-on-medical-research.html [accessed February 28, 2017].

Rosenthal, F.S., M. Kuisma, T. Lanki, T. Hussein, J. Boyd, J.I. Halonen, and J. Pekkanen. 2013. Association of ozone and particulate air pollution with out-of-hospital cardiac arrest in Helsinki, Finland: Evidence for two different etiologies. J. Expo. Sci. Environ. Epidemiol. 23(3):281-288.

Routledge, H.C., S. Manney, R.M. Harrison, J.G. Ayres, and J.N. Townend. 2006. Effect of inhaled sulphur dioxide and carbon particles on heart rate variability and markers of inflammation and coagulation in human subjects. Heart 92(2):220-227.

Samet, J.M., A. Rappold, D. Graff, W.E. Cascio, J.H. Berntsen, Y.C. Huang, M. Herbst, M. Bassett, T. Montilla, M.J. Hazucha, P.A. Bromberg, and R.B. Devlin. 2009. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. Am. J. Respir. Crit. Care Med. 179(11):1034-1042.

Sand, K., N.L. Eik-Nes, and J.H. Loge. 2012. Readability of informed consent documents (1987-2007) for clinical trials: A linguistic analysis. J. Empir. Res. Hum. Res. Ethics 7(4):67-78.

Sandman, P.M. 1987. Explaining risk to non-experts: A communications challenge. EP Dig. (October-December):25-29.

Schaumann, F., P.J.A. Borm, A. Herbrich, J. Knoch, M. Pitz, R.P.F. Schins, B. Luettig, J.M. Hohlfeld, J. Heinrich, and N. Krug. 2004. Metal-rich ambient particles (particulate matter 2.5) cause airway inflammation in healthy subjects. Am. J. Respir. Crit. Care Med. 170(8):898-903.

Schenker, Y., A. Fernandez, R. Sudore, and D. Schillinger. 2010. Interventions to improve patient comprehension in informed consent for medical and surgical procedures: A systematic review. Med. Decis. Making 31(1):151-173.

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Schneider, A., L. Neas, M.C. Herbst, M. Case, R.W. Williams, W. Cascio, A. Hinderliter, F. Holguin, J.B. Buse, K. Dungan, M. Styner, A. Peters, and R.B. Devlin. 2008. Endothelial dysfunction: Associations with exposure to ambient fine particles in diabetic individuals. Environ. Health Perspect. 116(12):1666-1674.

Shaaban, R., S. Kony, F. Driss, B. Leynaert, D. Soussan, I. Pin, F. Neukirch, and M. Zureik. 2006. Change in C-reactive protein levels and FEV1 decline: A longitudinal population-based study. Respir. Med. 100(12):2112-2120.

Shafiq, N., and S. Malhotra. 2011. Ethics in clinical research: Need for assessing comprehension of informed consent form? Contemp. Clin. Trials 32(2):169-172.

Shah, A.P., A.P. Pietropaoli, L.M. Frasier, D.M. Speers, D.C. Chalupa, J.M. Delehanty, L. Huang, M.J. Utell, and M.W. Frampton. 2008. Effect of inhaled carbon ultrafine particles on reactive hyperemia in healthy human subjects. Environ. Health Perspect. 116(3):375-380.

Shamoo, A.E., and D.B. Resnik. 2006. Strategies to minimize risks and exploitation in phase one trials on healthy subjects. Am. J. Bioeth. 6(3):W1-W13.

Silverman, D.T., C.M. Samanic, J.H. Lubin, A.E. Blair, P.A. Stewart, R. Vermeulen, J.B. Coble, N. Rothman, P.L. Schleiff, W.D. Travis, R.G. Ziegler, S. Wacholder, and M.D. Attfield. 2012. The diesel exhaust in miners study: A nested case-control study of lung cancer and diesel exhaust. J. Natl. Cancer Inst. 104(11):855-868.

Sin, D.D., L. Wu, and S.F. Man. 2005. The relationship between reduced lung function and cardiovascular mortality: A population-based study and a systematic review of the literature. Chest 127(6):1952-1959.

Slovic, P. 1987. Perception of risk. Science 236(4799):280-285.

Sparks, J. 2002. Timeline of Laws Related to the Protection of Human Subjects [online]. Available: https://history.nih.gov/about/timelines_laws_human.html [accessed May 25, 2016].

Speizer, F.E., and J.H. Ware. 2015. Exploring different phenotypes of COPD. N. Engl. J. Med. 373(2):185-186.

Spektor, D.M., M. Lippmann, G.D. Thurston, P.J. Lioy, J. Stecko, G. O'Connor, E. Garshick, F.E. Speizer, and C. Hayes. 1988. Effects of ambient ozone on respiratory function in healthy adults exercising outdoors. Am. Rev. Respir. Dis. 138(4):821-828.

Stafoggia, M., F. Forastiere, A. Faustini, A. Biggeri, L. Bisanti, E. Cadum, A. Cernigliaro, S. Mallone, P. Pandolfi, M. Serinelli, R. Tessari, M.A. Vigotti, and C.A. Perucci. 2010. Susceptibility factors to ozone-related mortality: A population based case-crossover analysis. Am. J. Respir. Crit. Care Med. 182(3):376-384.

Stahl, D.L., K.M. Richard, and T.J. Papadimos. 2015. Complications of bronchoscopy: A concise synopsis. Int. J. Crit. Illn. Inj. Sci. 5(3):189-195.

Stiegel, M.A., J.D. Pleil, J.R. Sobus, M.K. Morgan, and M.C. Madden. 2015. Analysis of inflammatory cytokines in human blood, breath condensate, and urine using a multiplex immunoassay platform. Biomarkers 20(1):35-46.

Stunkel, L., M. Benson, L. McLellan, N. Sinaii, G. Bedarida, E. Emanuel, and C. Grady. 2010. Comprehension and informed consent: Assessing the effect of a short consent form. IRB 32(4):1-9.

Sudore, R.L., C.S. Landefeld, B.A. Williams, D.E. Barnes, K. Lindquist, and D. Schillinger. 2006. Use of a modified informed consent process among vulnerable patients: A descriptive study. J. Gen. Intern. Med. 21(8):867-873.

Sun, Q., X. Hong, and L.E. Wold. 2010. Cardiovascular effects of ambient air pollution exposure. Circulation 121(25):2755-2765.

Tamariz, L., A. Palacio, M. Robert, and E.N. Marcus. 2013. Improving the informed consent process for research subjects with low literacy: A systematic review. J. Gen. Intern. Med. 28(1):121-126.

Tamura-Lis, W. 2013. Teach-back for quality education and patient safety. Urol. Nurs. 33(6):267-271.

Taylor, H.E., and D.E. Bramley. 2012. An analysis of the readability of patient information and consent forms used in research studies in anaesthesia in Australia and New Zealand. Anaesth. Intensive Care 40(6):995-998.

Thurston, G.D., K. Ito, R. Lall, R.T. Burnett, M.C. Turner, D. Krewski, Y. Shi, M. Jerrett, S.M. Gapstur, W.R. Diver, and C.A. Pope III. 2013. Mortality and long-term exposure to $PM_{2.5}$ and its components in the American Cancer Society's Cancer Prevention Study II Cohort. Pp. 127-166 in National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components. Research Report 177. Boston: Health Effects Institute [online]. Available: http://pubs.healtheffects.org/getfile.php?u=934 [accessed May 27, 2016].

Thurston, G.D., R.T. Burnett, M.C. Turner, Y. Shi, D. Krewski, R. Lall, K. Ito, M. Jerrett, S.M. Gapstur, W.R. Diver, and C.A. Pope III. 2016a. Ischemic heart disease mortality and long-term exposure to source-related components of U.S. fine air particle pollution. Environ. Health Perspect. 124(6):785-794.

Thurston, G.D., J. Ahn, K.R. Cromar, Y. Shao, R. Reynolds, M. Jerrett, C.C. Lim, R. Shanley, Y. Park, and R.B. Hayes. 2016b. Ambient particulate matter air pollution exposure and mortality in the NIH-AARP diet and health cohort. Environ. Health Perspect. 124(4):484-490.

Tong, H., A.G. Rappold, D. Diaz-Sanchez, S.E. Steck, J. Berntsen, W.E. Cascio, R.B. Devlin, and J.M. Samet. 2012. Omega-3 fatty acid supplementation appears to attenuate particulate air pollution–induced cardiac effects and lipid changes in healthy middle-aged adults. Environ. Health Perspect. 120(7):952-957.

Tornqvist, H., N.L. Mills, M. Gonzalez, M.R. Mills, S.D. Robinson, I.L. Megson, W. Macnee, K. Donaldson, S. Söderberg, D.E. Newby, T. Sandström, and A. Blomberg. 2007. Persistent endothelial dysfunction in humans after diesel exhaust inhalation. Am. J. Respir. Crit. Care Med. 176(4):325-326.

UNC (University of North Carolina at Chapel Hill). 2014. Human Research Protection Program: Standard Operating Procedures, April 24, 2014 [online]. Available: http://research.unc.edu/files/2014/05/2014-04-24-OHRE-Standard-Operating-Procedures-FINAL-CLEAN.pdf [accessed May 27, 2016].

U.S. Department of Education. 2012. Program for the International Assessment of Adult Competencies [online]. Available: http://nces.ed.gov/surveys/piaac/ [accessed November 15, 2015].

U.S. Department of State. 2016. Mission China: Shenyang- $PM_{2.5}$ [online]. Available: http://www.stateair.net/web/post/1/5.html [accessed April 7, 2016].

Wang, D.S., A.B. Jani, M. Sesay, C.G. Tai, D.K. Lee, K.V. Echt, M.G. Goodman, K.E. Kilbridge, and V.A. Master. 2015. Video-based educational tool improves patient comprehension of common prostate health terminology. Cancer 121(5):733-740.

Wanzer, M.B., A.M. Wojtaszczyk, J. Schimert, L. Missert, S. Baker, R. Baker, and B. Dunkle. 2010. Enhancing the "informed" in informed consent: A pilot test of a multimedia presentation. Health Commun. 25(4):365-374.

WHO (World Health Organization). 2001. Biomarkers in Risk Assessment: Validity and Validation. Environmental Health Criteria 222. Geneva: WHO. 238 pp [online]. Available: http://www.inchem.org/documents/ehc/ehc/ehc222.htm [accessed May 27, 2016].

Williams, P.R. 2004. Health risk communication using comparative risk analyses. J. Expo. Sci. Environ. Epidemiol. 14(7):498-515.

Young, S.S., and J.Q. Xia. 2013. Assessing geographic heterogeneity and variable importance in an air pollution data set. Stat. Anal. Data Min. 6(4):375-386.

Zanobetti, A., and J. Schwartz. 2008. Mortality displacement in the association of ozone with mortality: An analysis of 48 cities in the United States. Am. J. Respir. Crit. Care Med. 177(2):184-189.

Appendix A

Biographical Information on the Committee on Assessing Toxicologic Risks to Human Subjects Used in Controlled Exposure Studies of Environmental Pollutants

Robert A. Hiatt (chair) is professor and chair of the Department of Epidemiology and Biostatistics at the University of California, San Francisco (UCSF). He also is the associate director of the UCSF Helen Diller Family Comprehensive Cancer Center. In addition, Dr. Hiatt holds adjunct appointments as professor in the Division of Epidemiology at the University of California, Berkeley School of Public Health and as an adjunct investigator at the Division of Research at Kaiser Permanente Northern California in Oakland. From 1998 to early 2003, he was the first deputy director of the Division of Cancer Control and Population Sciences at the National Cancer Institute, where he oversaw cancer research in epidemiology and genetics, surveillance, and health services research. Dr. Hiatt has been the principal investigator of the Bay Area Breast Cancer and the Environment Research Center that is studying the influence of environmental factors on pubertal maturation as a window to understanding the causes of breast cancer. He is a past president of the American College of Epidemiology and the American Society for Preventive Oncology. He has served on several past Institute of Medicine (IOM) committees including the Committee on Breast Cancer and the Environment: The Scientific Evidence, Research Methodology, and Future Directions. Currently, Dr. Hiatt serves as a member of the National Academy of Medicine (NAM) Committee on the State of the Science in Ovarian Cancer Research and the National Research Council's Board on Environmental Studies and Toxicology. He received an M.D. from the University of Michigan and a Ph.D. in epidemiology from the University of California, Berkeley.

A. John Bailer is university distinguished professor and chair in the Department of Statistics, Scripps Research Fellow in the Scripps Gerontology Center, faculty affiliate of the Statistical Consulting Center, affiliate member of the Department of Biology, and affiliate member of the Department of Sociology and Gerontology at Miami University in Oxford, Ohio. His research interests include the design and analysis of environmental and occupational health studies and quantitative risk estimation. Dr. Bailer is a fellow of the American Statistical Association (ASA), a fellow of the Society for Risk Analysis, and a recipient of the ASA Statistics and the Environment Distinguished Achievement Medal. He has served on several National Research Council committees, including the Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, the Committee on Spacecraft Exposure Guidelines, the Committee to Review the OMB Risk Assessment Bulletin, and the Committee on Toxicologic Assessment of Low-Level Exposures to Chemical Warfare Agents. He also has served as a member of the Report on Carcinogens Subcommittee and the Technical Reports Review Subcommittee of the Board of Scientific Counselors of the National Toxicology Program. Dr. Bailer received a Ph.D. in biostatistics from the University of North Carolina at Chapel Hill.

Rebecca Bascom is a professor of medicine at the Milton S. Hershey Medical Center at Pennsylvania State University. As a specialist in pulmonary medicine, her primary clinical focus is caring for patients with serious diseases of the lung. Dr. Bascom has conducted research on lung diseases and inhalation toxicology, including a team analysis to evaluate the cardiorespiratory health effects on New York City police officers exposed during the 9/11 terrorist attack. She is committed to multidisciplinary translational

research, connecting basic scientists to her lung disease patients, and ensuring consistency with Penn State University research policies and procedures. She has enrolled patients with tobacco-related lung diseases in clinical trials for the past 10 years. Previously, she performed controlled human exposure studies using sidestream tobacco smoke and evaluated mechanisms of injury. She has served on several National Research Council committees, including the Committee on the Evaluation of the Department of Defense Comprehensive Clinical Evaluation Protocol, the Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, and the Committee on Health Effects of Indoor Allergens. In addition, Dr. Bascom served on the Institute of Medicine Committee on Scientific Standards for Studies on Modified Risk Tobacco Products. She trained in internal medicine, as well as pulmonary and critical care medicine at the Johns Hopkins Hospital. Dr. Bascom earned an M.D. from the University of Oregon Health Sciences Center and an M.P.H. in occupational medicine from the Johns Hopkins Bloomberg School of Public Health.

Larry R. Churchill (NAM) is the Ann Geddes Stahlman Professor of Medical Ethics at Vanderbilt University, with a primary appointment in the Department of Medicine, and secondary appointments in Philosophy and the Graduate Department of Religion. Prior to Vanderbilt, Churchill was at the University of North Carolina at Chapel Hill (UNC), where he served as Department Chair of Social Medicine, 1988-1998. He has played a major role in developing medical ethics and humanities programs at both UNC and Vanderbilt. Dr. Churchill's major research projects have been focused on justice and health policy, care of the dying, research with human subjects, and, most recently, the ethical features of routine medical care. Dr. Churchill has served on numerous Institutional Review Boards (IRBs) and Data Safety Monitoring Committees and has published widely on the ethics of human-subjects research. He served on the Institute of Medicine committee issuing the report on *Complementary and Alternative Medicine in the United States* in 2005 and was the principal author for the ethics chapter in that study. In 1991 Churchill was elected to membership in the NAM, and has been a Fellow of The Hastings Center since 2000. His most recent books are *Healers: Extraordinary Clinicians at Work* (2012), and *What Patients Teach: The Everyday Ethics of Health Care* (2013), both from Oxford University Press. He received a Ph.D. in religion from Duke University.

Kenny S. Crump is an independent consultant. Previously, he was a principal with Environ Corporation. He has over 35 years of experience in assessing risks related to exposure to toxic materials. He has served on science advisory boards of the Environmental Protection Agency, the National Center for Toxicological Research, the Mickey Leland National Urban Air Toxics Research Center, and the National Institute of Environmental Health Sciences. His research interests are in the areas of biostatistics, health risk assessment, and analysis of epidemiologic data. Statistical models for assessing risk developed by Dr. Crump have been widely used by regulatory agencies and private groups. These include the Linearized Multistage Model and the benchmark methodology. Dr. Crump has participated in risk assessments of many substances, including asbestos, benzene, manganese, and mercury. He was previously a member of the National Research Council's Diesel Impacts Study Committee, the Committee on Risk Analysis Issues and Reviews, the Committee on Risk Assessment Methodology, and the Committee on Institutional Means for Assessment of Risks to Public Health. He recently served on the Institute of Medicine's Committee to Evaluate Potential Exposure to Agent Orange/TCDD Residue and Level of Risk of Adverse Health Effects for Aircrew of Post-Vietnam C-123 Aircraft. Dr. Crump received a Ph.D. in mathematics from Montana State University.

Daniela B. Friedman is professor and chair in the Department of Health Promotion, Education, and Behavior at the Arnold School of Public Health of the University of South Carolina. She is also a core faculty member of the university's Statewide Cancer Prevention and Control Program. Dr. Friedman uses both qualitative and quantitative methods to evaluate how people access, understand, and use information on disease risk and prevention. Her research examines individual and social influences on health comprehension, and she studies a variety of innovative strategies for the development and delivery of accurate, lan-

guage-appropriate, and culturally sensitive health information. She received a Ph.D. in health studies and gerontology from the University of Waterloo in Canada.

Diane R. Gold is a professor in the Department of Environmental Health in the School of Public Health at Harvard University, professor of medicine at Harvard Medical School, and associate physician at Brigham and Women's Hospital. Dr. Gold's research focuses on the relationships between environmental exposures and the incidence or severity of respiratory diseases, including asthma. The environmental exposures considered include indoor allergens, such as fungi, smoking, and outdoor ozone and particles. She investigates the environmental exposures that may explain socioeconomic, cultural, and gender differences which have been observed in asthma severity. These include perinatal exposures and family stress as well as exposure to the allergens and pollutants mentioned above. She is also interested in the cardiopulmonary effects of particles on the elderly and she has collaborated in research involving controlled human exposures. Dr. Gold served on the Institute of Medicine's Committee on an Assessment of Asthma and Indoor Air Quality. She received an M.D. from the University of Connecticut School of Medicine.

Lewis R. Goldfrank (NAM) is Herbert W. Adams Professor of the Department of Emergency Medicine at New York University, and medical director of the New York City Health Department's Poison Center. Dr. Goldfrank has worked at the Bellevue Hospital Center and New York University's Medical Center for more than 30 years. He has served on multiple IOM committees, including the Committee on Personal Protective Equipment for Healthcare Workers in the Workplace Against Novel H1N1 Influenza A. Dr. Goldfrank also served on the NAM Board on Health Sciences Policy. He is a long-standing member of NAM's Forum on Medical and Public Health Preparedness for Catastrophic Events. He received an M.D. from the University of Brussels Medical School. Dr. Goldfrank was elected to NAM in 1996.

Nancy E. Lane (NAM) is an Endowed Professor of Medicine and Rheumatology at the University of California at Davis. She is also director of the Center for Musculoskeletal Health and Aging Research and director of the K12 National Institutes of Health (NIH) Building Interdisciplinary Research Careers in Women's Health program. She is principal investigator of the NIH-funded Program on Sex Differences in Musculoskeletal Diseases Across the Lifespan at the University of California at Davis School of Medicine. Dr. Lane was president of the board of the United States Bone and Joint Decade and she co-led the International Bone and Joint Decade Conference in Washington, DC. She received an M.D. from the University of California at San Francisco School of Medicine. Dr. Lane was elected to the National Academy of Medicine in 2013.

Morton Lippmann is a research professor in the Department of Environmental Medicine at New York University's School of Medicine. Dr. Lippmann's research has involved a series of studies that seek to identify the physical and chemical components of airborne particles responsible for observed health effects, including subchronic exposures to concentrated fine particles in a mouse model of atherosclerosis, and observational studies of human populations for association of acute and cumulative responses to exposures in the general atmospheric environment. Dr. Lippmann has served on several National Research Council committees, including the Committee on Air Quality in Passenger Cabins of Commercial Aircraft, the Committee on Toxicology, and the Committee on Research and Peer Review in the U.S. Environmental Protection Agency (EPA). He received a Ph.D. in environmental health science from New York University.

Murray A. Mittleman is an associate professor in the Department of Epidemiology at the Harvard School of Public Health and an associate professor of medicine at the Harvard Medical School. He is the director of the Cardiovascular Epidemiology Research Unit at the Beth Israel Deaconess Medical Center; vice-chair of the Committee on Clinical Investigations, which serves as the Institutional Review Board, at the Beth Israel

Deaconess Medical Center; and chair of the Master of Public Health program at the Harvard School of Public Health. Dr. Mittleman's applied research focuses on behavioral and environmental determinants of acute cardiovascular events and their prognosis. He received an M.D. from McGill University and an M.P.H. and Dr.P.H. in epidemiology from the School of Public Health at Harvard University.

Philip Needleman (NAS/NAM) was elected to the National Academy of Sciences in 1987 and to the Institute of Medicine in 1993. He was a professor and chair of the Department of Pharmacology at Washington University Medical School (1976-1989) and Chief Scientist and head of R&D for Monsanto/Searle/Pharmacia (1989-2003). He was interim president of the Donald Danforth Plant Science Center (2009-2010) and interim president and CEO of the St. Louis Science Center (2010-2011). He has served on the NAS council, chaired NAS Section 23, and served on the NAS Division of Earth and Life Studies. Dr. Needleman received a Ph.D. in pharmacology from the University of Maryland at College Park.

Robert F. Phalen is co-director of the Air Pollution Health Effects Laboratory, professor in the Department of Medicine, and professor in the Center for Occupational and Environmental Health at the University of California, Irvine (UCI) School of Medicine. In 1972, Dr. Phalen joined the College of Medicine at UCI to establish the Air Pollution Health Effects Laboratory, which conducts studies relating to the toxicology of air pollutants. His research areas include lung modeling for predicting doses from inhaled particles, lung morphometry for growing mammals, health effects of inhaled air pollutants, and applied aerosol physics. He chaired the IRB at UCI for 7 years. He served on the National Research Council's Committee on Animal Models for Testing Interventions Against Aerosolized Bioterrorism Agents. Dr. Phalen received a Ph.D. in biophysics, with specialization in inhalation toxicology, from the University of Rochester.

Margaret Foster Riley is a professor of law in the School of Law, professor of public health sciences in the School of Medicine, and professor of public policy in the Batten School of Leadership and Public Policy at the University of Virginia. Her research focuses on human-subjects research law and ethics, biotechnology, health care regulation, and food and drug law. She serves as chair of the university's Embryonic Stem Cell Research Oversight Committee and as legal advisor to the Health Sciences Institutional Review Board, and is a member of the executive committee of the Center for Health Policy. Prior to joining the University of Virginia, Ms. Riley was an associate with Pepper Hamilton & Scheetz in Philadelphia, where she worked primarily in complex securities, commercial, and mass tort litigation. Prior to that position, she was a litigation associate with Rogers & Wells in New York. She served on the National Research Council's Committee on Revisions to the Common Rule for the Protection of Human Subjects in Research in the Behavioral and Social Sciences, and has advised numerous committees of the Institute of Medicine and the Virginia Bar. Ms. Riley received a J.D. from Columbia University.

Hwashin H. Shin is a research scientist in the Environmental Health Science and Research Bureau of Health Canada. She is also an adjunct professor in the Department of Mathematics and Statistics of Queen's University in Ontario, Canada. Previously, she was a research associate at the Institute of Population Health at the University of Ottawa. Her research areas include environmental public health risk models, epidemiology, and experimental optimal design. Dr. Shin's recent research topics include bias correction in estimation of public health risk attributable to short-term air pollution exposure, and the systematic review and meta-analysis of outdoor fine particles and strokes. Dr. Shin is a member of the Outdoor Air Pollution Committee of Global Burden Disease. She received a Ph.D. in statistics from Queen's University.

Appendix B

Public Information-Gathering Sessions

COMMITTEE ON ASSESSING TOXICOLOGIC RISKS TO HUMAN SUBJECTS USED IN CONTROLLED EXPOSURE STUDIES OF ENVIRONMENTAL POLLUTANTS

PUBLIC INFORMATION-GATHERING SESSION
June 1, 2015

National Academy of Sciences Building
2101 Constitution Avenue, NW
Washington, DC 20418

Opening Remarks from Robert Hiatt and Introduction of Committee Members

EPA Research Overview
Robert Kavlock (Deputy Assistant Administrator for Science, EPA/ORD)

Foundations of the NAS Charge: Ethical Issues in Foreseeable Risk,
Toby Schonfeld (Director, EPA Program in Human Research Ethics and Oversight; EPA Human Subjects Research Review Official)

Conduct, Process, and Outcomes of Exposure Studies,
Wayne Cascio (Director, Environmental Public Health Division, National Health Environmental Effects Research Laboratory, EPA/ORD)

David Diaz-Sanchez (Chief, Clinical Research Branch, National Health Environmental Effects Research Laboratory, EPA/ORD)

EPA Human Subjects Regulatory Requirements
Dan Nelson (Director, Human Research Protocol Office, National Health Environmental Effects Research Laboratory, EPA/ORD)

Summary and Wrap-Up
Toby Schonfeld

Questions from Committee

Opportunity for Public Comment

End of Session

Appendix B

PUBLIC INFORMATION-GATHERING SESSION
August 24, 2016

Webinar

Opening Remarks from Robert Hiatt (Committee Chair) and Introduction of Committee Members

Presentation from Steve Milloy
Publisher, JunkScience.com

Presentation from John Dunn
Civilian Faculty Emergency Medicine
Carl R. Darnall Army Medical Center

Presentation from Stanley Young
Fellow, American Statistical Association and the American Association for the Advancement of Science

Questions from Committee

Opportunity for Public Comment
James Enstrom (UCLA and Scientific Integrity Institute)
Albert Donnay (Johns Hopkins Center for Sleep Disorders)

End of Session

Appendix C

Assessment of Eight Controlled Human Exposure Studies

The U.S. Environmental Protection Agency (EPA) provided eight CHIE studies to the committee for its consideration.[1] The studies were selected from among the CHIE studies at the EPA Human Studies Facility that were active at some point in time from January 2009 to October 2016. See Table C-1. The eight studies are:

> Cardiopulmonary Responses to Exposure to Ozone and Diesel-Engine Exhaust with Moderate Exercise in Healthy Adults (DEPOZ)
>
> Effects of Sequential Exposure to Nitrogen Dioxide and Ozone in Healthy Adult Human Volunteers (ENDZONE)
>
> Epigenetic Effect Modifications with Ozone Exposure on Healthy Volunteers (GEMINOZ)
>
> Mechanisms by which Air Pollution Particles Exacerbate Asthma in Older Adults with Mild Asthma (KINGCON)
>
> Cardio-protective effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles (OMEGACON)
>
> The Interaction of Social Factors with Air Pollution (SOZIAL)
>
> Effects of Wood Smoke Particles on Influenza-Induced Nasal Inflammation in Normal Volunteers (WOODSIE)
>
> Physiologic Changes in Adults with Metabolic Syndrome Exposed to Concentrated Ultrafine Chapel Hill Air Particles (XCON)

The committee used the following considerations:

1. Research Question:
   - Is the research question well focused?
   - Is there a clear hypothesis that is testable?

2. Background, Gap Analysis, and Rationale
   - Range and variation of pollutant exposures in the United States and perhaps elsewhere.
   - Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.).
   - Current air quality standard and the relevance for future reviews of the standard.

[1] An application for institutional review board approval and a consent to participate in a research study for each CHIE study was provided by EPA on November 19, 2014. Publications resulting from those eight studies are Devlin et al. 2014 and Ghio et al. 2012 for XCON; Tong et al. 2012, 2015 for OMEGACON; Madden et al. 2014; Stiegel et al. 2015; Stiegel et al. 2016 for DEPOZ.

- Critical toxicologic pathways and evidence of perturbations.
- Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.).
- Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
- Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
- Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
- Research goal regarding the
  - Relationship between physiologic function and pollutant exposure,
  - Biologic plausibility and/or mechanisms of air pollution health effects,
  - Interpretation of effects observed in toxicologic or epidemiologic studies, and
  - Other: Statistical analysis plan in detail at proposal stage.

3. Study Design
   Does the design of the study adequately reflect the information uncertainty being addressed?

4. Subject Selection
   Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?

5. Experimental Methods
   Is the study method appropriate?

6. Exposure Protocol
   Was the choice of the study's exposure concentration and duration appropriate?

7. End points
   - Was the choice of study end points appropriate for the experimental question?
   - Were the time points for measurement appropriate?

8. Analysis
   Was the statistical analysis appropriate?

9. Interpretation and Generalizability of the Findings (expected or reported)
   - What are the strengths and limitations of the study?
   - What are the major findings of the study?
   - What are the remaining uncertainties?
   - Describe how the findings have or may contribute to the following aspects:
   - Quantification of the relationship between physiologic function and pollutant exposure,
   - Understanding biologic plausibility of effects of concern,
   - Increased ability to interpret effects observed in toxicologic or epidemiologic studies, and
   - Other: Contributions of publications out of the study.

Controlled Human Inhalation-Exposure Studies at EPA

TABLE C-1 Controlled Inhalation Exposure Studies in EPA Human Studies Facility[a]

Study Name[b]	Title & IRB Number	Exposure	# Subjects (may include some who did not complete all study arms)	# Exposures to Active Pollutant	# Exposures to Control (Clean Air)
ASTHMACON	Physiological Changes on Mild to Moderate Asthmatics Exposed to Concentrated Chapel Hill Ambient Particles (99-EPA-80)	Clean air and $PM_{2.5}$ CAPS (avg 80.1 ug/m^3)	18	15	17
CAPTAIN	Cardiopulmonary Effects of Exposure of Healthy Older GSTM1 Null and Sufficient Individuals to Concentrated Ambient Air Particles (11-1807)	Clean air and $PM_{2.5}$ CAPS (avg. 190.3 ug/m^3)	18	13	18
CHAPS	Respiratory Effects of short term low level Chlorine Gas Exposure (05-EPA-535)	Clean air and 0.4 ppm chlorine gas	17	17	16
DEPOZ	Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults (09-1344)	Clean air, diesel exhaust (300 ug/m^3, and O_3 (0.3 ppm)	21	52	18
ENDZONE	Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers (13-0459)	Clean air, O_3 (0.3 ppm, and NO_2 (0.5 ppm)	33	149	61
FLAIR (UNC)	Effects of diesel exhaust particles on influenza-induced nasal inflammation in allergic rhinitis and non-allergic individuals (07-1064)	Clean air, diesel exhaust	62	32	30
GEMINOZ	Epigenetic effect modifications with ozone exposure on healthy volunteers (Non-twin) (13-3697)	Clean air and O_3 (0.3 ppm)	13	13	13
GARBOZ (UNC)	Genetic Susceptibility to Ozone-Induced Bronchial Airway Inflammatory Responses in Humans (02-CEMLAB-616)	0.4ppm O_3	102	103	10
GLUTOZ (UNC)	Glutathione S Transferase M1 (GSTM1) genotype associated susceptibility to airway response to ozone in volunteers with mild asthma (GCRC-2371)	0.4ppm O_3	25	25	0
KINGCON	Mechanisms by which air pollution particles exacerbate asthma in older adults with mild asthma (06-0548)	Clean air and $PM_{2.5}$ CAPS (avg 181.4 ug/m^3)	14	14	14
LAMARK	Epigenetic effects of diesel exhaust and ozone exposure (09-1625)	Clean air, O_3 (0.3 ppm) and diesel exhaust (300 ug/m^3)	37	69	34
LOCONOZ	Pulmonary responses to exposure to low concentration ozone for 6.6 hours with moderate exercise in healthy adults (07-1811)	Clean air and O_3 (0.06 ppm and 0.08 ppm)	61	92	60
MOSES (UNC)	Multicenter Ozone Study of Elderly Subjects (11-0803)	Clean air and O_3 (0.07 ppm and 0.12 ppm)	31	31	59

(Continued)

TABLE C-1 Continued

Study Name[b]	Title & IRB Number	Exposure	# Subjects (may include some who did not complete all study arms)	# Exposures to Active Pollutant	# Exposures to Control (Clean Air)
OBOZ (UNC)	Effect of obesity on ozone induced airway inflammation (05-1644)	Clean air and 0.4 ppm O_3	44	41	44
OMEGA	Pilot to examine increasing doses of diesel exhaust, subsequently converted to OMEGACON	Clean air and diesel exhaust (100, 200, 300 ug/m^3)	6	18	0
OMEGACON	Cardio protective effects of Omega-3 fatty acids supplementation in healthy older subjects exposed to air pollution particles (07-0190)	Clean air and $PM_{2.5}$ (avg 278 ug/m^3)	35	30	35
SMOKEY	Effects of wood stove emissions in adults (08-0334)	Clean air and wood smoke emissions (avg 493 ug/m^3)	12	11	12
SOZIAL	The Interaction of Social Factors with Air Pollution (13-1644)	Clean air and O_3 (0.3 ppm)	46	44	42
TROPICOZ	Interaction Effects of Temperature and Ozone (11-0772)	Clean air and O_3 (0.3 ppm)	16	16	16
WOODSIE (UNC)	Effects of Wood Smoke particles on Influenza-Induced Nasal Inflammation in Normal Volunteers (13-3076)	Clean air and wood smoke emissions (approx 500 ug/m^3)	39	20	19
XCON	Physiological Changes in Adults with Metabolic Syndrome Exposed to Concentrated Ultrafine Chapel Hill Air Particles (04-1677)	Clean Air and ultrafine PM (110,000-330,000 particles/cc)	XCON 1 18	18	15
			XCON 2 23	22	22

[a]The studies were active at some point from January 2009 to October 2016.
[b]"UNC" means the study was conducted in the EPA Human Studies Facility but initiated or led by UNC investigators, many with EPA collaboration.
Source: EPA, unpublished material, submitted November 2, 2016.

CARDIOPULMONARY RESPONSES TO EXPOSURE TO OZONE AND DIESEL EXHAUST WITH MODERATE EXERCISE IN HEALTHY ADULTS (DEPOZ)

Is the research question well focused?

This study focused on the joint effect on lung function of diesel-engine exhaust (DE) exposure and ozone (O_3), and on the effect of prior exposures to DE, O_3, and DE+O_3 on the subsequent response to O_3 alone. These effects were measured by sampling forced vital capacity (FVC) and forced expiratory volume for 1 second (FEV_1). In addition to samples of FVC and FEV_1, the protocol called for monitoring of heart rate variability and blood pressure, and collection of samples of blood, saliva, and urine, and included information on cardiovascular function. However, there was no clear indication of how these data would be statistically analyzed or otherwise used to study cardiovascular responses to O_3 and DE. The published report from this study (Madden et al., 2014) does not report on cardiovascular effects.

Controlled Human Inhalation-Exposure Studies at EPA

Is there a clear hypothesis that is testable?
There were three specific hypotheses tested by this study: It was hypothesized that an exposure to DE with O_3 (day 1) or DE exposure (day 1) given prior to O_3 exposure (day 2) would not induce a significant decrement in lung function in healthy young adults as a group relative to O_3 alone; that an O_3 exposure (on day 2) after the DE and O_3 coexposure (day 1) would cause significant cardiopulmonary responses in healthy young adults; and that 2 consecutive days of O_3 exposure would affect cardiovascular responses. The first two hypotheses involving pulmonary responses could be tested using the data collected on FVC and FEV_1.

The hypothesis concerned with cardiovascular responses is not as specific as those involving lung function and the protocol does not state how this hypothesis would be tested. The statistical power calculation is based only pulmonary function. Thus, it is not clear that the hypothesis involving cardiovascular responses was testable.

Range and variation of pollutant exposures in the United States and perhaps elsewhere.
The proposal does not contain a review of pollution levels in various locations. In the information provided to participants, some largely anecdotal information regarding typical DE levels in various situations are provided (truck drivers, miners, near busy intersections), but complete references are not provided.

Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.).
This study focused on the joint effect on lung function of DE and O_3 exposure. People of all ages are exposed to these contaminants.

Current air quality standard and the relevance for future reviews of the standard.
Air quality standards related to DE and O_3 are not discussed in the proposal. Also, there is no description of how the results of this study could affect air quality standards, other than a general statement that "[t]he data obtained from this study will contribute to the overall assessment of air pollution effects in the U.S. and thereby may influence future health policy."

Critical toxicologic pathways and evidence of perturbations
For DE exposures, evidence is presented of adverse cardiopulmonary effects including premature mortality, cardiopulmonary problems including infections, exacerbation of asthma symptoms, and heart attacks. For O_3 exposures, evidence is presented for decrements of lung function and an influx of neutrophils and other markers of inflammation. Studies are cited that indicate persons with a glutathione-*S*-transferase Ml (GSTM1) null genotype may be particularly susceptible to the effects of O_3 exposure on lung function.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.).
There are no documents cited that state a need for this research. It is stated without citation that it is not known whether coexposure to both O_3 and DE, as would occur when exposed to polluted ambient air, can induce additive or synergistic effects, and also whether exposure to DE, or DE with O_3, can alter a subsequent exposure to O_3.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
This study only investigated responses to O_3 and DE under very specific and not very realistic conditions. There still remain questions regarding responses that would be expected under variable and more realistic conditions.

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
This study could reinforce the indications that exposure to O_3 can have reversible effects on pulmonary function. It could also provide information on whether the combination of DE and O_3 exposure would have a greater than additive effect and provide some information on the level of such an effect.

Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
The relationship between the results of this study and how the results might be used in setting regulations is not discussed directly. The study does address whether persons with a GSTM1 null genotype may be a sensitive subpopulation that is particularly susceptible to the effects of O_3 and DE on lung function.

Research goal regarding the...
Relationship between physiologic function and pollutant exposure
The research goals including comparing short-term lung function resulting from simultaneous exposure to O_3 and DE to that resulting from separate exposures to these substances and also to compare the effect of prior exposures to O_3 and DE to lung function after exposure to O_3 only.

Biologic plausibility and or mechanisms of air pollution health effects
One research goal was to elucidate the effect of the GSTM1 null genotype on the effects of O_3 exposure on lung function.

Interpretation of effects observed in toxicologic or epidemiologic studies
It had been observed in a mouse model that both DE and O_3 were needed to increase lung resistance, which was not observed with individual pollutant exposures (Madden et al. 2014). DEPOZ investigated whether similar responses would be present in humans.

Does the design of the study adequately reflect the information uncertainty being addressed?
The goals of the study involved clarification of short-term effects of exposure regimens, as opposed to possible long-term effects. The study design was appropriate for this limited goal.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?
The subjects in this study were selected from among healthy relatively young adults (men and women between the ages of 18 and 55 years). Therefore, their responses will possibly not be reflective of responses that might occur in the elderly or the very young or in those who already have compromised health.

Is the study method appropriate?
The study method is appropriate for testing the narrow hypotheses being tested.

Was the choice of the study's exposure concentration and duration appropriate?
The rationale for the exposure concentration and duration are not clearly stated in the proposal. There is no clear description of the rationale for the target exposures selected (0.3 ppm O_3, and 300 $\mu g/m^3$ DE). Several studies are cited that exposed volunteers to 300 $\mu g/m^3$ DE. A number of studies involving O_3 exposures are cited but this information is not tied to the exposure level of 0.3 ppm used in this study. Apparently this team has experience with exposing O_3 to volunteers at levels around 0.3 ppm. But this experience is not clearly stated in the application or referred to in support the 0.3 ppm exposure level.

Also, there is no mention of the type of diesel engine that would be used to generate the exhaust to which the volunteers would be exposed to. Diesel technology has undergone important changes in recent years

and DE emissions have changed both quantitatively and qualitatively (Hesterberg et al., 2011 McClellan et al. 2012, Khalek et al. 2011, 2015). Also, diesel emissions are composed of gases and particulate of various sizes. The protocol does not state what fraction of this complex mixture will be used to quantify DE. For a full characterization of DE exposure, information on the diesel engine to be used to generate the DE should have been provided in the proposal along with specific information on how DE is to be measured. The manufacturer and model of the diesel engine used to generate the DE was provided in the published paper (but not in the protocol), although no characterization of the emissions from this engine were provided (Madden et al., 2014).

Was the choice of study end points appropriate for the experimental question?
The principle end points were indicators of lung function as measured by FEV_1 and FVC. These end points were sufficient to answer questions about effects on lung function. However, they provided little direct information on effects on cardiovascular function.

Were the time points for measurement appropriate?
FVC_1 and FEV were measured immediately following the completion and hourly thereafter for 4 hours, and again 20 hours following the completion of exposure. This was sufficient to indicate the trend in re-bound in FVC_1 measurements, and FVC_1 measurements had returned to baseline or nearly so after 20 hours (Madden et al., 2014).

Was the statistical analysis appropriate?
The data obtained in the study were analyzed using a repeated measures analysis of variance (ANOVA) parametric test. This type of test is appropriate for a randomized crossover design like that used in this study. The statistical power calculation indicated that 14 subjects would provide 80% power for detecting a 10% decrease in FEV_1 from O_3 exposure. This calculation seems appropriate. However, this power calculation applies only to the FEV_1 data and does not provide information on the power from analysis of cardiovascular data.

What are the strengths and limitations of the study?
The study design and group size was adequate to study the limited hypotheses of the study. Like most studies of this type it involved a limited number of healthy individuals exposed to a very small range of exposure conditions. Consequently, it would be problematic to use the results from this study to predict responses in the population at large.

What are the major findings of the study?
Results from this study suggest that altered respiratory responses to the combination of O_3 and DE exposure can occur in a greater than additive manner, and O_3-induced lung function decrements can be greater with a prior exposure to DE compared to a prior exposure to filtered air (Madden et al. 2014).

What are the remaining uncertainties?
Like all studies of limited and prescribed exposures to volunteers, many questions remain. The study exposed healthy volunteers between the ages of 18 and 55. Effects upon the very young and old and upon those with existing health conditions were not investigated. The study involved a limited number of exposure conditions, none of which are typical of real-world exposures, so using the results of the study to predict responses in real-world situations would be problematic.

Quantification of the relationship between physiologic function and pollutant exposure.
The study provided quantitative information on the physiologic response to exposure to O_3, DE, and O_3+DE, and response to O_3 following earlier exposures O_3, DE, and O_3+DE, in a few healthy volunteers, under specific exposure conditions. It could be problematic to extrapolate these quantitative responses to more general situations.

Understanding biologic plausibility of effects of concern.
The results of this study reinforced the biologic plausibility of effects upon lung function from exposure to O_3 and O_3+DE.

Increased ability to interpret effects observed in toxicologic or epidemiologic studies.
The results from this study could possibly provide reinforcement to interpretations of effects observed other studies.

EFFECTS OF SEQUENTIAL EXPOSURE TO NITROGEN DIOXIDE AND OZONE IN HEALTHY ADULT HUMAN VOLUNTEERS (ENDZONE)

Is the research question well focused? Yes.

Is there a clear hypothesis that is testable? Yes.

Range and variation of pollutant exposures in the United States and perhaps elsewhere. Good.

Relevance of the condition chosen for study in the general population (e.g., age, disease, etc).
Appropriate.

Current air quality standard and the relevance for future reviews of the standard.
Good.

Critical toxicologic pathways and evidence of perturbations.
Adequate, in consideration of the paucity of relevant prior published research.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.). Good.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered. Good.

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
Yes, it would generate useful and interesting additional knowledge. However, it would be inadequate, with regard to level of certainty about the real-world effects of sequential inhalation exposures to O_3 and NO_2.

Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
Not applicable insofar as known sensitive subpopulations are not being studied.

Research goal regarding the...
Relationship between physiologic function and pollutant exposure.
Limited, insofar as only one concentration and one exposure duration are specified.

Biologic plausibility and or mechanisms of air pollution health effects.
It could provide valuable increments on the biologic mechanisms contributing to the effects of inhaled O_3 and NO_2 alone, and especially on any additional responses resulting from sequential exposures.

Interpretation of effects observed in toxicologic or epidemiologic studies

a. New interpretations of the toxicologic effects of inhaled O_3 are unlikely, since these effects of short-term exposures to O_3 alone are already well studied and described;
b. New interpretations of the toxicologic effects of inhaled NO_2 are possible, but also unlikely, since there has been virtually no consistency in reported effects of short-term inhalation exposures to NO_2 alone in previous studies with single concentrations in the same range;
c. New interpretations of the acute epidemiologic associations of inhaled O_3 are unlikely, since these associations are already well studied and described;
d. New interpretations of any acute epidemiologic effects of inhaled NO_2 alone are unlikely given that any previously reported associations of NO_2 with either acute or chronic inhalation exposures are as, or more, likely to be due to copollutants; and
e. New interpretations of the toxicologic effects of sequential inhalation exposures to O_3 and NO_2 are unknowable in advance, but could be interesting and important if they occur.

Other.
Direct coherence of physiologic responses to inhaled O_3 and NO_2 from toxicologic and epidemiologic studies, and their concentration–response relationships, as they may affect the selection of short-term National Ambient Air Quality Standards (NAAQS), is unlikely, because controlled human exposures are (a) almost always at constant concentrations for relatively short time intervals, while ambient air exposures vary during the day, extend over longer time intervals, and vary with location within a community; (b) almost always to O_3 or NO_2 alone, while ambient air exposures always include other gaseous and particulate matter (PM) components that can produce the same or similar responses; and (c) O_3 and NO_2 are index pollutants for the NAAQS for photochemical oxidants and NO_x, respectively. Epidemiologic responses may be due, in part, to peroxides and/or nitric acid in the ambient air as well as O_3 and NO_2.

Does the design of the study adequately reflect the information uncertainty being addressed?
No, with respect to the temporal sequences of the exposures. For a study of the physiologic effects of sequential exposures of inhalation exposures to O_3 and NO_2, it was disappointing that there was no justification provided for the temporal sequences. As noted in the submission, the real-world sequential exposures involve peak morning exposures to NO_2 followed by peak early afternoon exposures to O_3. Why then select the second of the sequential 2-hour controlled inhalation exposures 24 hours later? The premise of the statement that the effects of the ambient air exposures to O_3 or NO_2 may be severe and delayed seem odd in view of the absence of literature documenting severe effects.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?
No, but the population to be studied is the correct one for this exploratory study of sequential exposures.

Is the study method appropriate? Yes.

Was the choice of the study's exposure concentration(s) and duration appropriate?
They were reasonable.

Was the choice of study end points appropriate for the experimental question? Yes.

Were the time points for measurement appropriate?
Yes, but measurements at additional time points could have been very informative.

Was the statistical analysis appropriate? Yes.

What are the strengths and limitations of the study?
Summarized above.

EPIGENETIC EFFECT MODIFICATIONS WITH OZONE EXPOSURE ON HEALTHY VOLUNTEERS (GEMINOZ)

Is the research question well focused?
The purpose of this protocol is to assess whether epigenetic factors in (1) healthy individuals or (2) individuals with the same genetic makeup (i.e., identical twins) make a person more or less responsive to inflammation following ozone exposures. Yes, the research question is well focused.

Is there a clear hypothesis that is testable? Yes there is a clear hypothesis that is testable.

Range and variation of pollutant exposures in the Untied States and perhaps elsewhere.
The proposal does not contain a review of pollution levels in various locations. In the information provided to participants on air pollution and heart and pulmonary disease it does not state it.

Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.).
Yes. This study focused on individuals 18-40 years of age, healthy, nonsmoking, and from the mid-Atlantic twins registry and healthy age-similar controls.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.).
Yes. This is a study of exposure to ozone and clean air, and exposure to ozone and its detrimental effects on the pulmonary system are well documented. Responses of primary interest are pulmonary: lung function, lung inflammation, and epigenetic changes as evaluated in bronchoalveolar lavage.

Relationship between physiologic function and pollutant exposure.
The research goals include comparing short-term lung function. Epigenetic responses obtained from the alveolar cell after pollutant exposure. The hypothesis is that pollutant exposure will modulate the epigenome and this will trigger gene expression that could result in proteins produced that alter pulmonary function. The study will be done in normal controls, and in monozygotic (MZ) twins to evaluate the biologic changes (epigenome) after pollutant exposure.

Biologic plausibility and or mechanisms of air pollution health effects
That there is biologic plausibility response to ozone in the lung may depend on the epigenome and this crossover study in MZ twins may confirm that observation or hypothesis and will lead to new research and possible risk factor identification of ozone and lung injury.

Does the design of the study adequately reflect the information uncertainty being addressed?
The goals of the study involved clarification of short-term effects of lung inflammation in MZ twins exposed in a crossover design to either clean air, then ozone, or in reverse. The study subjects are between the ages of 18 and 40 years and there are no significant health concerns. The study design was appropriate for this limited goal.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?
The subjects in this study were selected from among MZ twin healthy adults (men and women between the ages of 18 and 40 years).

Is the study method appropriate?
The study method is appropriate for testing the narrow hypotheses being tested.

Was the choice of the study's exposure concentration and duration appropriate?
The rationale for the exposure concentration and duration are similar to their published protocols from this group. The subjects will all be exposed to clear air then will be randomized to ozone or clean air, then after 13 days there will be a crossover. Responses of interest include lung inflammation, lung function, and epigenetic changes evaluated by bronchopulmonary lavage. The end point was chosen because other work has shown that epithelial cells lining the airways are the first target of ozone and respond by making proinflammatory cytokines such as IL-6 and IL-8. Epigenetic changes are dependent on tissue type, and airway epithelial cells can be obtained from brush biopsies during bronchoscopy and assayed for epigenetic changes. The study's aim will determine if baseline epigenetic profiles between subjects are associated with responsiveness to ozone and if ozone exposure itself causes acute changes in a subject's epigenome.

Was the statistical analysis appropriate?
Yes. The analysis will estimate the within-twin associations for twin subjects and overall associations for nontwin subjects between ozone response and gene-specific methylation. They propose to take a pathway approach where they estimate associations between inflammatory pathway–specific combinations of gene-specific methylation scores most associated with ozone responsiveness. Specifically they will look at the percent change of FEV_1, percent PMNs (type of white blood cell), IL-8, and PG-2 present in the lung between baseline and exposure conditions. For twins, the outcomes will be contrasted with those of the corresponding twin.

What are the strengths and limitations of the study?
This study's goal is to detect within-twin correlation for twin subjects and overall associations for nontwin subjects between methylation and ozone susceptibility, and all power calculations were made under the assumption of two-sided tests at a significance level, global at the pathway level, of 0.05 response, combined with a conservative Bonferroni correlation for multiple tests across genes within a pathway. For the inflammatory pathway of 20 genes for n=50 twin pairs or 50 nontwin subjects, p = 0.025, 80% power, to detect a correlation of 0.43 between ozone, measured continuously, and methylation. The power calculations were made under the assumption of two-sided tests, global at the pathway level.

Limitations of this study are the MZ twins, in which the environmental exposure is the only difference between them, so the association is probably going to be as good as it could get. This study will be very challenging to duplicate in the general population; however, these results if definitive could lead the way to new treatments to reduce the toxicities of air pollution.

The study design and group size was adequate to study the limited hypotheses of the study. Like most studies of this type it involved a limited number of healthy individuals exposed to a very small range of exposure conditions. Consequently, it would be problematic to use the results from this study to predict responses in the population at large.

MECHANISMS BY WHICH AIR POLLUTION PARTICLES EXACERBATE ASTHMA IN OLDER ADULTS WITH MILD ASTHMA (KINGCON)

Is the research question well focused? Yes.

Is there a clear hypothesis that is testable? Yes.

Range and variation of pollutant exposures in the United States and perhaps elsewhere. Adequately described.

Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.). Appropriate importance.

Current air quality standard and the relevance for future reviews of the standard.
Current air quality standards and the relevance to future studies. The citations state that particulate matter is an important part of air pollution and concentrations of exposure in the study are cited. But the standard is neither stated nor reviewed other than stating the exposure in the study will not exceed the total exposure encountered in 24 hours on a typical urban smoggy day. Future reviews of the standard are not addressed.

Critical toxicologic pathways and evidence of perturbations. Discussion appropriate.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.). Acceptable, extensive citations.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
There is very limited discussion of remaining information needs after current study. Forthcoming studies are not addressed except for the storage of blood RNA/DNA sample.

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
Yes, further details on pulmonary function of aging asthmatics will be determined but the level of certainty is limited.

Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
Nothing is addressed. Although standards for environmental exposures are not cited the implication of these studies has great importance for standard setting. This presentation to the Institutional Reveiw Board (IRB) would be clearer if the principles and background were presented in the study submission.

Does the design of the study adequately reflect the information uncertainty being addressed? Include consideration of the outcome measures (effects which may occur readily and be mild) relative to the key health effects of concern for the studied air pollutant (which may be severe and delayed).
The study design reflects information uncertainty. This is a study of short-term exposure addressing acute phase reactants, acute oxidative stress, and immediate physiologic responses. The outcome measures will need evaluation with regard to long-term potential toxic effects from single exposures, cumulative effects, chronic manifestations, and specific interactions of exposures. The effect of age and genetic susceptibility will be better addressed. The study of specific inhalants for those with more consequential and different airway diseases may lead to more specific and refined study of current air quality standards. There are few long-term or broad hypotheses for future work offered in the submission.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?
The specificity of age, pulmonary function, chronic diseases, and drug or medication use are well presented. There is confusion between Exclusion Criterion 1—stating severe asthma and Exclusion Criterion 4—stating moderate to severe asthma, although elsewhere under subjects it is stated only patients with mild

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asthma are included. These restrictive entry criteria lay the groundwork for future studies to define populations at other levels of risk.

Is the study method appropriate?
Yes, the methods are rigorous and appropriate.

Was the choice of the study's exposure concentration and duration appropriate?
The authors do not offer current air quality standard data, suggesting only the maximum exposure is comparable to a smoggy urban day. In addition, they suggest neither the risk of >600 $\mu g/m^3$ particulate mass levels nor the reason for a 10-minute delay prior to termination of the study. It is not clear whether since the concentration may exceed 600 $\mu g/m^3$ (by an unknown amount) for 16 minutes prior to study termination that the risk is adequately addressed.

Was the choice of study end points appropriate for the experimental question? Yes.
Were the time points for measurement appropriate?
These end points are chosen for immediate evaluation and evaluation at 24 hours. This study exposure is not expected to have long-term effects and was not designed to assess that risk or effect.

Was the statistical analysis appropriate? Yes.

Interpretation and generalizability of the findings (expected or reported)
There is one spelling error— "immunodeficiency" and one misleading abbreviation—the term "chronic respiratory diseases" stands alone—but it must include chronic asthma (mild). It should be rewritten as "chronic respiratory diseases including moderate to severe asthma."

CARDIO-PROTECTIVE EFFECTS OF OMEGA-3 FATTY ACIDS SUPPLEMENTATION IN HEALTHY OLDER SUBJECTS EXPOSED TO AIR POLLUTION PARTICLES (OMEGACON)

Is the research question well focused?
The purpose of this study was to examine the health effects of fine and ultrafine particulate matter (PM<2.5) exposure on the cardiovascular system and examine whether omega-3 fatty acids supplementation pretreatment would attenuate the adverse cardiovascular effects. A strong positive outcome would provide support for the EPA to advocate a prevention strategy. Overall, the research question is well focused as laboratory data support the role of omega-3 fatty acids as modulating a toxic response.

Is there a clear hypothesis that is testable?
There is a clear hypothesis that fine and ultrafine ambient PM (PM< 2.5) exposures alter the outcome of adverse cardiac events, especially on the cardiac autonomic function and systemic inflammation. Another goal was to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular (CV) effects of PM exposure. It is known that omega-3 polyunsaturated fatty acids have several potential cardioprotective effects including antiarrhythmic, antithrombotic, anti-inflammatory, and lowering lipid levels.

Range and variation of pollutant exposures in the United States and perhaps elsewhere.
The proposal does not contain a review of pollution levels in various locations in the information provided to participants on air pollution and heart and pulmonary disease.

Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.).
This study focused on middle aged individuals 50-75 years of age. The age is appropriate for heart disease, and both males and females were studied. O_2 saturation of >94% was used, which is normal O_2 saturation.

Current air quality standard and relevance for future review of standard N/A

Critical toxicologic pathways and evidence of perturbations
For PM exposures, evidence is presented of adverse cardiopulmonary effects including premature mortality, cardiopulmonary problems including infections, exacerbation of asthma symptoms, and heart attacks. For PM exposures, evidence is presented for decrements of lung function and an influx of neutrophils and other markers of inflammation. Studies are cited that indicate persons with a GSTM1 null genotype may be particularly susceptible to the effects of PM exposure on lung function.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.).
The background documentation cites literature on PM exposure, air pollution, and elevated risk of cardiovascular diseases. Also, epidemiologic studies that show that ASCVD is higher in areas with high air pollution. Similarly there are data cited that omega-3 fatty acids reduce heart disease, arrhythmias, sudden death, and sudden cardiac death. Data support that air pollutants change the autonomic function in the heart, and this may be mediated by oxidative stress. The respiratory effects of PM are well known, but the CV ones are not. The goals of this study were to determine if fish oils/omega-3 fatty acids would reduce or mitigate respiratory and cardiovascular effects of PM.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
This study only investigated responses to PM under very specific and not very realistic conditions. After 4 weeks of fish or olive oil supplementation, subjects were exposed to PM, then end points were neutrophil counts, lung function, inflammation markers, heart rate variability, brachial artery diameter (flow mediated dilation [FMD]), change in blood vasoactivators, and coagulation factors. Secondary end points included pulmonary function tests and endothelial cell function, and peripheral venous blood markers. Exploratory end points included inflammatory cytokines and GSTM1 allele variants. There still remain questions regarding responses that would be expected under variable conditions, and after longer-term exposure.

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
This study could reinforce that the pulmonary and cardio vascular effects of acute PM exposure can be modified with fatty acids (FAs) or olive oil (OO), if study durations were longer and a dose response was evaluated.

Research goal in the regulatory context of providing public health protection, including identification and protection of sensitive populations.
Good. The study focused on an at-risk, sensitive population for heart disease with the goal of determining if FA or OO supplementation would prevent PM-induced stress on the cardiovascular system.

Research Goal regarding the...
Relationship between physiologic function and pollutant exposure.
The research goals included comparing short-term lung function, and changes in cardiovascular function (heart rate variation and pulmonary function, FMD) with and without fish oil or olive oil supplementation. Also, second goal older subjects with GSTM1 genotype will have a lower risk of CV events than

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GSTM1 null genotype when exposed to PM and have worse outcomes than GSTM1 positive genotypes and could benefit more from fish oil.

Biologic plausibility and/or mechanisms of air pollution health effects.
There is biologic plausibility that fish oils can reduce the CV effects and inflammatory pulmonary effects of PM. In addition, the GSTM1-null variant subjects have worse outcomes after PM, and may have a more significant reduction in their response with fish oil or olive oil.

Interpretation of effects observed in toxicologic or epidemiologic studies.
It had been observed in clinical studies that PM affects pulmonary and cardiovascular function and fish oils/olive oil reduce CV events through a number of pathways including antioxidation and anti-inflammatory pathways. This information provided the rationale for these studies.

Does the design of the study adequately reflect the information uncertainty being addressed?
The goals of the study involved clarification of short-term effects of exposure regimens, as opposed to possible long-term effects. The study design was appropriate for this limited goal.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?
The subjects in this study were selected from among healthy adults (men and women between the ages of 50 and 75 years). Therefore, their responses will be reflective of responses that might occur in the elderly who already have compromised health function that could include cardiovascular or pulmonary diseases.

Is the study method appropriate?
The study method is appropriate for the narrow hypotheses being tested.

Was the choice of the study's exposure concentration and duration appropriate?
The rationale for the exposure concentration and duration are not clearly stated in the proposal. The study subjects were exposed to clear air for 2 hours on one day and then air with fine and ultrafine PM (PM< 2.5) for 2 hours on the second day in an exposure chamber after the supplementation. There is no clear description of the rationale for the target exposures selected, that of concentrated airborne particulate matter (CAP) (mean 253±16 $\mu g/m^3$) for 2 hours. Several studies are cited that exposed for epidemiology literature and animal studies; however, this exposure was not derived from literature. Apparently this team has experience with exposing to volunteers at levels around MEAN 253±16 $\mu g/m^3$ of fine particulate matter. A study by Romieu et al. (2005) studied omega-3 fatty acid to prevent heart disease associated with particulate matter. But this experience is not clearly stated in the application or referred to in support of that exposure level.

Was the choice of study end points appropriate for the experimental question? Yes.

Were the time points for measurement appropriate? Yes, but measurements at additional time points could have been informative.

Was the statistical analysis appropriate?
The data obtained in the study were analyzed using ANOVA parametric test for continuous variables, and rank sum tests for noncontinuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and postexposure. This type of test is appropriate for a randomized double-blinded study like that used in this study. The statistical power calculation indicated that 30 subjects (15 per group) would provide 80% power for detecting a 0.13 unit change in brachial artery ultrasound (BAU) diameter. This calculation seems appropriate. However, this power calculation applies only to the BAU diameter and does not provide information on the power from the other end points in the study.

What are the strengths and limitations of the study?
Summarized above. The study design and group size were adequate to study the limited hypotheses of the study. Like most studies of this type it involved a limited number of healthy individuals exposed to a very small range of exposure conditions. Consequently, results of this study should be interpreted cautiously and not necessarily used to predict responses in the population at large.

What are the major findings of the study?
There were two publications (Tong et al., 2012, 2015).

1. Heart rate variability (HRV) was significantly elevated in the OO group immediately after PM exposure whereas the omega-3 groups showed no significant change. The response was quite clear. QT interval as prolonged by OO but not fish oil (FO) but the actual response was quite modest. The most striking finding was that pollution exposure in the OO group markedly elevated very low density lipoprotein (VLDL) and triglycerides whereas FO effectively lowered both. Thus, FO blunted the negative heart rate variability and QT prolongation caused by exposure to particulate pollutants.
2. This study published in 2015 was from the same protocol but with a different outcome. The key finding was that dietary supplementation with OO but not FO clearly blunted the negative impact of particulates of FMD. In addition, the OO group exhibited increased levels of the fibrinolysis marker tPA after particulate exposure. The results from all the other assays were largely inconclusive when comparing the groups.

What are the remaining uncertainties?
Like all studies of limited and prescribed exposures to volunteers, many questions remain. The study exposed healthy volunteers between the ages of 50 and 75 years. Effects upon the very young and old and upon those with existing health conditions were not investigated. The study involved a fixed exposure, none of which are typical of real-world exposures, so using the results of the study to predict responses in real-world situations would be problematic.

Describe how the findings have or may contribute to the following aspects:
Quantification of the relationship between physiologic function and pollutant exposure.
The study provided quantitative information on the cardiovascular and pulmonary and inflammatory responses to fine particulate matter, PM < 2.5μm, in individuals aged 50 to 75 years under specific exposure conditions. It could be problematic to extrapolate these quantitative responses to more general situations.

Understanding biologic plausibility of effects of concern.
The results of this study reinforced the biologic plausibility of effects upon particulate matter mean 253±16 $\mu g/m^3$ and the cardiovascular, thrombolytic systems observed in toxicologic or epidemiologic studies.

Increased ability to interpret effects observed in toxicologic or epidemiologic studies. Not clear.

Other: Contributions of publications out of the study. None.

OMEGACON Publication Reviews

Purpose of Study
To examine the health effects of fine and ultrafine particulate matter (PM<2.5) exposure on the cardiovascular system and examine whether omega-3 fatty acid supplementation pretreatment would attenuate the adverse cardiovascular effects. A strong positive outcome would provide support for the EPA to advocate a prevention strategy.

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Randomized, double-blind study.
The subjects were exposed to clean air on the first day then concentrated ambient fine and ultrafine PM on the second day after the dietary supplementation. Possible health effects of acute 2-hour exposure to air pollution particles include chest pain, mild dyspnea, headache, cough/wheeze, various adverse cardiovascular effects such as increase in heart rate, and decreases in HRV.

Two publications in *Environmental Heath Perspectives* resulted from this study.

Tong et al. 2012. Omega-3 Fatty Acid Supplementation Appears to Attenuate Particulate Air Pollution–Induced Cardiac Effects and Lipid Changes in Healthy Middle-Aged Adults.

Objective:
Study effects and protective potential of omega-3 fish oil (FO) versus olive oil (OO) control on cardiac function and lipid pretreatment in response to acute air pollution exposure.

Design:
29 patients, 16 FO, 13 OO-3 g/day oil for 28 days. Patients first exposed to filtered air, then next day to 30-fold concentrated ambient air (300 $\mu g/m^3$ particulate) for 2 hours. Ambulatory EKG measurements for heart rate variability (HRV), QT changes, ventricular polarization. Venous blood measurements for plasma fatty acids, triglycerides, VLDL, HDL.

Results:
The plasma fatty acid composition reflected the diet with the FO patients having substantially elevated EPA (6X), and DPA (2X)-docosa-pentaenoic acid and somewhat lower omega-6 arachidonic acid.

Cardiac results:
HRV was significantly elevated in the OO group immediately after PM exposure whereas the omega-3 group showed no significant change. The response was quite clear. QT interval was prolonged by OO but not FO but the actual response was quite modest. The most striking finding was that pollution exposure in the OO group markedly elevated VLDL and triglycerides, whereas FO effectively lowered both. Thus, FO blunted the negative heart rate variability and QT prolongation caused by exposure to particulate pollutants.

Discussion:
The discussion evoked many literature citations and implications that the current data responses implied autonomic nervous system changes—but there were no actual data to prove that point. In fact the effects of FO on QT were quite modest, and the pollution elevation of triglycerides and VLDL was consistent with the previous literature. The authors' suggestion that the particulate-exposed cardiac change was a potential mechanism of atherosclerosis is unwarranted. Furthermore, it is not surprising that omega-3 fatty acids reduce the pollution elevated triglycerides. Triglyceride lowering has firmly been shown with FO in many studies. The negative cardiac effects of pollution and the positive triglyceride effect of FO were previously established.

Tong et al. 2015. Dietary Supplementation with Olive Oil or Fish Oil and Vascular Effects of Concentrated Ambient Particulate Matter Exposure in Human Volunteers.

The publication evaluates the potential beneficial vascular effect of 4-week FO or OO dietary supplementation in 42 patients exposed to 2 hours of $PM_{2.5}$ particulate pollution.

The primary physiologic measurements were reactive hyperemia measured as flow mediated dilation (FMD), brachial artery diameter, and blood pressure. ELISA assays of venous blood measured numerous coagulation factors, lipid and vascular markers.

Results:
The key finding was that dietary supplementation with OO but not FO clearly blunted the negative impact of particulates of FMD. In addition, the OO group exhibited increased levels of the fibrinolysis marker tPA after particulate exposure. The results from all the other assays were largely inconclusive when comparing the groups.

Discussion:
The elaborate discussion of the literature tried unsuccessfully to implicate endothelial dysfunction. Indeed, two major, well-documented mediators of endothelia-vascular changes, nitric oxide and prostacyclin, were not measured.

Interestingly, there was no mention of the 2012 paper in the discussion. In that report the same authors found that OO supplementation had the negative impact by elevating the heart rate variability and prolonged QT interval caused by acute pollution exposure, i.e., omega-3 was protective. On the other hand, the 2015 paper proposed that OO was protective against the reactive hyperemia produced by pollutants and not FO. The differing effects of FO of various cardiovascular responses to pollution do not warrant, at this point, advocating the protective potential of FO.

In both papers the authors pointed out "conclusions derived from the small numbers of participants included in the study may not be applicable to the population as a whole. Furthermore, the modest sample size and the number of secondary endpoints measured could inflate the significance of the findings."

Opinion:
The authors are quite correct about the limitations of such a small study. In addition, acute 2-hour exposure is insufficient to project about the anticipated population exposure, which is chronic. These papers were published in specialized journals. Key findings were predictable. The limitations and inconsistencies (especially in the blood marker studies) in the data would suggest publication in a rigorous cardiovascular medical journal seems highly unlikely.

Overall:
The justification of such human experiments considering the obvious limitations in design is unclear. The negative effects of the pollution particulates are well documented. Any safety risk in small, limited trials seems unwarranted. Overall, these studies were hypothesis generating and large trials with greater exposure times would be required to determine clinical utility.

THE INTERACTION OF SOCIAL FACTORS WITH AIR POLLUTION (SOZIAL)

Is the research question well focused? Yes.

Is there a clear hypothesis that is testable? Yes.

Range and variation of pollutant exposures in the United States and perhaps elsewhere.
Usual and experimental levels of ozone are clearly described.

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Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.). Eligibility of only younger individuals (18-33 years) reduces the likelihood of adverse reactions, but may not be relevant to older individuals who are an important target population to consider for these air pollution exposures.

Current air quality standard and the relevance for future reviews of the standard.
The current standard is less than 300 ppb ozone and this protocol follows that standard.

Critical toxicologic pathways and evidence of perturbations.
The concept and physiologic role of stress pathways and allostasis are well described.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.).
Appropriate literature cited.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
No comment.

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
The study should result in important new information that takes into account the characteristics of exposed populations and not just the physiologic or toxicologic responses.

Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
Although this study may provide information on persons with different perceived levels of stress, it does not really allow recommendations to be delivered to any particular population subgroup (e.g., disadvantaged populations of low socioeconomic position).

Research goal regarding the...
Relationship between physiologic function and pollutant exposure. Yes.

Biologic plausibility and or mechanisms of air pollution health effects. Yes.

Interpretation of effects observed in toxicologic or epidemiologic studies. Partially.

Does the design of the study adequately reflect the information uncertainty being addressed? Yes, except for some lack of clarity in their randomization procedure. There were two arms described: one with low PPS and the other with high PPS. However, it was not clear whether the randomization to ozone exposure or to clean air (placebo) was carried out *within* arms or strata of PPS or for all volunteers together. It should have been within strata.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?
As above, it includes persons ages 18-33 years and may miss effects likely in older groups with more compromised lung function or ability to deal with stress. However, the likelihood of adverse reactions in an older group is likely increased.

Is the study method appropriate? Yes.

Was the choice of the study's exposure concentration and duration appropriate? Yes.

Was the choice of study end points appropriate for the experimental question? Yes.

Were the time points for measurement appropriate? Yes.

Was the statistical analysis appropriate?
The planned analysis seems appropriate, but there were no actual analyses to review.

What are the strengths and limitations of the study?
As stated above the restriction to younger ages limits the generalizability to the full population age range.

EFFECTS OF WOOD SMOKE PARTICLES ON INFLUENZA-INDUCED NASAL INFLAMMATION IN NORMAL VOLUNTEERS (WOODSIE)

Is the research question well focused?
The research question is well focused.

Is there a clear hypothesis that is testable?
There is a clear and testable hypothesis: exposure to wood smoke particles enhances influenza virus–induced granulocyte and NK cell activation via hyaluronic acid–mediated effects on IFNg production.

Range and variation of pollutant exposures in the United States and perhaps elsewhere.
Wood smoke is an increasingly important source of ambient particulate matter in the United States. Outdoor sources include landscape fires (67,774 wildfires involving 9,326,238 acres in the United States in 2012) while indoor sources include the use of wood for indoor heating or recreation/ambience, structural fires, and intrusion of outdoor source wood smoke.

Wood smoke is also an international health concern with 2 billion people using biomass (including wood) for indoor heating and cooking. In some areas, indoor-generated smoke is an important contributor to outdoor particulate air pollution.

Relevance of the condition chosen for study in the general population (e.g. age, disease, etc.).
The condition chosen for study, influenza virus infection, is highly relevant because influenza infections are an important cause of morbidity and mortality in the United States and worldwide. The finding that wood smoke alters influenza infection would have broad public health implications. The physiologic and biomarker changes shown in this study could potentially be used for population-based studies.

Current air quality standard and the relevance for future reviews of the standard.
The current primary air quality standard for $PM_{2.5}$ is 12 $\mu g/m^3$ as an annual mean, averaged over 3 years and a 24-hour standard of 35 $\mu g/m^3$ (98th percentile averaged over 3 years). The current primary air quality standard for PM_{10} is 150 $\mu g/m^3$ which is a 24-hour averaging time not to be exceeded more than once per year on average over 3 years.

The concentration used in this study is 500 $\mu g/m^3$ for 2 hours' duration, and is justified based on the measurement of similar levels of particulate matter sometimes seen in homes using wood as their main energy source.

Critical toxicologic pathways and evidence of perturbations.
A strength of the study is the focus on critical pathways for viral host defense and seeking evidence of perturbations with wood smoke exposure.

Document citations that support a need for this research (i.e. strategic plan, consensus statements, etc.).

World Health Organization (WHO 2015)

Responsive to EPA Clean Air Research Multi-Year Plan 2008-2012 (EPA 2008).

APM 5: Identify new biomarkers of exposure and/or effects to specific PM components and associated gases

APG 3: Elucidate the susceptibility and vulnerability factors that increase risk with adverse health outcomes associated with air pollutants.

APG 6: Evaluate the importance of key biologic pathways in explaining how air pollutants cause adverse health outcomes.

APM 22: Identify the mechanisms by which air pollutants cause adverse health effects.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
The IRB submission does not review epidemiologic and animal/*in vitro* toxicologic data (this is not normally required).

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
Pathways of toxicity and biologic plausibility.

Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
Wood smoke exposure studies might help identify sensitive subgroups.

Does the design of the study adequately reflect the information uncertainty being addressed?
The study design adequately reflects the information uncertainty being addressed. There is a clean air comparator allowing each subject to serve as his/her own control. A strength of the design is the inclusion of repeated time points, and sampling of nasal lavage fluids, nasal biopsy material, and blood.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to project?
The study is approved for 40 subjects, with 14 enrolled prior to the renewal request. Healthy nonsmoking adults age 18-40 are the study population. The air pollution standard applies to this group of individuals. The standard also extends to sensitive subgroups including children, the elderly, and people with respiratory diseases. It is possible that differences in immune function in these groups would result in differences in host response.

Is the study method appropriate?
The study methods are appropriate, with use of standardized dosing with live attenuated influenza virus. The range of inflammatory end points includes mediators and cell-based assays.

Was the choice of the study's exposure concentration and duration appropriate?
The exposure protocol was based on the study of Ghio et al. in which healthy adult volunteers were exposed to 500 $\mu g/m^3$ wood smoke particles (WSPs) for 2 hours with exercise. The investigator indicates the exposure is lower than that for forest firefighters, those living in areas near forest fires or agricultural burning, or people living in developing countries using biomass for cooking.

Was the choice of the study end points appropriate for the experimental question?
The choice of end points is appropriate for the pathways under investigation. A strength of the study is the partnering with other laboratories, leveraging the study results to address additional end points (granulocyte activation and lipid mediator activation) that are highly relevant to the toxicologic pathways involved in this response.

Were the time points for measurement appropriate?
Time points are appropriate (0, 1, 2, 7-10, and 21-28 days), covering the anticipated duration of infection and assessing acute and subacute time points.

Was the statistical analysis appropriate?
A sample size justification is provided and is acceptable. It is based on the area under the curve for nasal lavage fluid gamma interferon based on their previously published study of diesel exposure. Absent previous wood smoke exposure data, this seems a reasonable surrogate.

What are the strengths and limitations of the study?
Strengths of the study are the public health importance of the research question, the experimental design with a robust array of end points and time points.

Describe how the findings have or may contribute to the following aspects:
Quantification of the relationship between physiologic function and pollutant exposure.
Physiologic function (and biomarkers) are evaluated.

Understanding biologic plausibility of effects of concern.
The investigators have examined these pathways looking at other toxicants (e.g., tobacco smoke).

Increased ability to interpret effects observed in toxicologic or epidemiologic studies.
Will provide biologic plausibility as well as indicate end points for use in epidemiologic studies. Will also assist in interpretation of findings from animal/toxicologic studies.

PHYSIOLOGICAL CHANGES IN ADULTS WITH METABOLIC SYNDROME EXPOSED TO CONCENTRATED ULTRAFINE CHAPEL HILL AIR PARTICLES (XCON)

Is the research question well focused?
The investigators state that the research question is to examine the acute health effects of concentrated ambient ultrafine (UF) particulate matter (PM) exposure in patients with metabolic syndrome. The outcomes they are studying are not, however, clinical health effects, but rather biologic and physiologic responses that may, if associated with the exposure, lend biologic plausibility to observational studies finding associations of UF with clinical outcomes.

This particular subgroup (a subgroup with metabolic syndrome) is at risk of developing cardiovascular disease (CVD) or type 2 diabetes mellitus (DM), independent of PM exposure. Clinical CVD and DM have been shown to increase susceptibility to clinical health effects of ambient particle pollution. Some (but not all) studies suggest that metabolic syndrome and its components may increase susceptibility to

inflammatory or physiologic effects of pollution. The purpose of this study, as stated in the IRB application,[2] is

> … "to examine the acute health effects of concentrated ambient UF PM exposure in patients with metabolic syndrome… Our hypothesis is that PM exposure in this population will result in changes in endothelial response as assessed by flow-mediated dilatation of the brachial artery and various heart rate variability and blood endpoints. This study and similar studies of susceptible populations are needed to provide the EPA with information regarding the health risks associated with ambient levels of UF PM" (p. 7).

The IRB application for XCON also states, "The results from this human study will provide information data that assist the EPA in determining whether or not to retain the current standard on PM" (p. 15).

EPA's 2011 policy assessment for the review of the PM NAAQS indicates that the agency considered "whether there is support to consider standards with a different size fraction and/or distinct standards focused on regulating a specific $PM_{2.5}$ component or group of components associated with any source categories of fine particles" (EPA 2011, pp. 1-16). However, EPA staff concluded that there is insufficient information at this time to consider a separate indicator for ultrafine particles.

Also, the potential role of ultrafine particles in causing adverse health effects, relative to $PM_{2.5}$, may inform the development of mitigation approaches. EPA's Clean Air Research Multi-Year Plan 2008-2012 states (EPA 2008):

> … "recent concerns regarding traffic exposures have prompted exposure profiles for ultrafine PM emissions relative to distance from roadways. There now exist some measures that tie to freeways, traffic volume and vehicle type. ORD has particular interest in the effect of various mitigation methods, especially as they relate to indoor penetration values. Building type and ventilation appear to be major factors in penetration of ultrafine and coarse mode PM, as well as oxidant gases, but appear to be less significant (on a relative basis) for fine PM and less reactive gases" (p. 41).

Is there a clear hypothesis that is testable?
The hypothesis is that UF particular matter (PM) exposure in this population will result in changes in endothelial response as assessed by flow-mediated dilation of the brachial artery and various electrophysiologic outcomes (e.g., heart rate variability) and blood end points.

The study used a design of two exposure treatments x two sequences x two periods. Each exposure was separated by at least 2 weeks. One consideration regarding the testability of the hypothesis is whether the wait period between exposure treatments was sufficient to ensure washout of the previous exposure, and yet short enough to minimize the potential effect of confounders. To avoid the potential carryover effect, a panel for the Food and Drug Administration (FDA) has recommended that the 2 x 2 crossover design not be used in drug evaluation (Kuehl, 1994). Although it is not considered to be a critical shortcoming for this study, it is important that the choice of the washout period not be routine, but instead be based on a consideration of the specific aspects of a particular experiment.

Range and variation of pollutant exposures in the United States and perhaps elsewhere.
The proposal does not contain a review of UF levels in various locations. It mentions a number of times that the 600,000 particles/cc is similar to what an individual might experience driving in a heavy traffic highway in Los Angeles, but no other range or variation information is discussed.

[2]Application for IRB approval of human subjects research for XCON. EPA, unpublished material, November 19, 2014.

Relevance of the condition chosen for study in the general population (e.g., age, disease, etc.).
Appropriate. This study is focused on evaluating 34 subjects with metabolic syndrome between the ages of 25 and 70 years. This age range is a good reflection of the population with metabolic syndrome; however, it usually has a higher prevalence after the age of 50 years (44% in this study). Also it was reported that about 24% of the American adult population meets the definition of metabolic syndrome, which supports selected target subgroups (non-negligible size of susceptible subgroups).

Current air quality standard and the relevance for future reviews of the standard.
Air quality standards related to UF PM exposure and lung function are not discussed in the proposal. Also, there is no description of how the results of this study could affect air quality standards. The current standards are based on $PM_{2.5}$ or PM_{10}. Since the EPA is asking whether there should be a standard based on UF, this study can provide an answer to the necessity of future EPA standard for UF PM but not to the standard. The EPA should clarify whether it is conducting a study to clarify where efforts for regulation should focus, in terms of regional sources, even if the regulation may not directly relate to standard setting.

Critical toxicologic pathways and evidence of perturbations.
The background/rationale for the toxicologic pathways and evidence of perturbations for UF PM at current or below EPA air quality standards and cardiovascular disease, both morbidity and mortality are provided. There is a good description of why the particles cause organ toxicity and the leading hypothesis that very small particle size can move deep into the lung tissue with inspiration, and cause greater tissue damage or move more easily into the circulation. Identification of subsets in our U.S. population that are at higher risk for toxicity from PM (the elderly, individuals with cardiopulmonary diseases, and people with diabetes) is discussed, as well as the biologic underpinnings of potential susceptibility of people with metabolic syndrome to pollution.

Document citations that support a need for this research (i.e., strategic plan, consensus statements, etc.).
Good. The document supports a need for this research by demonstrating it is part of the EPA strategic plan, and by statistics about the increasing prevalence of metabolic syndrome in the United States, how this syndrome is associated with increased CV disease, and endothelial dysfunction manifesting in a pro-inflammatory and prothrombotic state due to increased production of inflammatory cytokines and C reactive protein. Since endothelial dysfunction and inflammation are toxicities of PM of air pollution, the rationale to support this research is acceptable. Previously, the investigators have evaluated CAP effects on endothelial function in subjects with diabetes. Evaluating the UF PM effects on endothelial response and blood end points in individuals with metabolic syndrome is supported adequately by citations.

Remaining information needs after current and forthcoming epidemiologic and animal/*in vitro* toxicologic data are considered.
The XCON IRB application[3] indicated that:

> "Human exposure studies are essential in order to determine the effects of a 'real-world' UF PM exposure in a potentially susceptible population without overt disease. In vitro and in vivo instillation studies are limited by the uncertainty associated with extraction of particles from filters or other substrates as it is not clear if all components get extracted or if the extraction process alters the chemistry of the particles. Furthermore, particles tend to agglomerate during extraction and their altered size range results in potential deposition in the lung at sites different from where 'real-world' unextracted particles would deposit when inhaled. Thus it is important to use 'real-world' particles

[3]Application for IRB approval of human subjects research for XCON. EPA, unpublished material, November 19, 2014.

whenever possible. A new generation instrument is now available that allows concentration of particles in the UF to "low-fine" range (0.03-0.25 μm)" (pp. 9-10).

Additional knowledge and/or level of certainty that this controlled human exposure study would provide.
This is not clear, as the purpose of this study is to evaluate the acute health effects (biologic and physiologic) of UF PM exposure in patients with metabolic syndrome (MS). This study has a specific focus, as it is only planning to study 30 subjects with MS, and the end point changes in endothelial response assessed by FMD of BA and HRV and blood end points. This study is part of the charter of the EPA to study susceptible populations and determine health risks with ambient levels UF PM.

If the investigators find associations of UF PM with the subclinical outcomes they are measuring, this may lend biologic plausibility to observational studies linking UF with clinical health outcomes, and may inform regulation if it is feasible for the EPA to regulate UF, which fall off rapidly as distance from traffic increases. If they do not find associations, this may be because (1) the population is too small; (2) the UF specific to Chapel Hill are not toxic (limited generalizability); or (3) UF does not cause the effects that they are measuring.

The conclusions reported in the publication are as follows:

- Exposure to concentrated ambient ultrafine particles (UCAPS) does not cause changes in brachial artery diameter or blood pressure.
- Exposure to UCAPS causes changes in cardiac repolarization and heart rate variability.
- Exposure to UCAPS causes changes in vascular markers of inflammation and fibrinolysis.

In comparison to $PM_{2.5}$, whether or not the findings above are related to the small size or number of UF is not fully discussed.

Research goal in the regulatory context of providing public health protection, including the identification and protection of sensitive subpopulations.
As mentioned above, results of this study will inform considerations of whether a UF-specific standard is warranted. This study is directed to protection of a sensitive subpopulations—individuals with metabolic syndrome.

Research goal regarding the...
Relationship between physiologic function and pollutant exposure.
The toxicologic data information on how UF PM may influence biologic and physiologic outcomes and the endothelium is described. Since individuals with MS have high risk of CVD, the need to determine how UF exposure affects this subpopulation is important.

Biologic plausibility and or mechanisms of air pollution health effects.
Previous data from earlier studies are discussed to demonstrate the toxicity in the lung and endothelial cells from UF PM, and the association with CVD. Also, the discussion of subpopulations that are at increased risk for CVD from air pollution is given.

Interpretation of effects observed in toxicologic or epidemiologic studies.
The background epidemiology and genetic epidemiology to support performing this study are adequate.

Other: Statistical analysis plan in detail at proposal stage.
The substantial involvement of a statistician at the proposal stage is critical in ensuring the appropriate design of experimental questions and statistical questions according to the research questions or goals.

Specifically at the proposal stage, a simulation and a pilot test are very useful for planning and setting experimental design. A few scenarios are usually possible based on previous studies or known facts.

Study Design

Does the design of the study adequately reflect the information uncertainty being addressed?

This is a double-blinded study in which each participant will be exposed to filtered clean air and air containing concentrated UF particles in randomized order. Through both blinding and randomization this study could avoid bias in testing.

The study has a two-treatment, two-sequence, and two-period crossover design, where the treatment effects are intended not to be confounded with the effects of sequences or periods. It was not reported whether baseline observations were taken prior to any treatment to assess potential carryover effects.

The study design also included repeated measurements over a 24-hour time span. This enables the investigation if the UF PM effect is immediate, delayed, or remained within a day. Thus, the proposed study design was reasonable but further investigation on time-related effect of UF was not done.

Is the health status of the study subjects reflective of the sensitive subgroups that the relevant air pollution standard is intended to protect?

Metabolic syndrome and its components are risk factors for CVD and type-2 DM. Those study subject characteristics are considered to be reflective, albeit indirectly, of sensitive subgroups. If there were an interest in directly studying subjects known to be at higher risk of clinical cardiovascular outcomes with acute air pollution exposures to these particles (e.g., those with overt diabetes or clinical cardiovascular disease), other study designs, such as community-based, repeated-measures, observational studies, would be more appropriate.

The subjects in this study were selected for metabolic syndrome in men and women between the ages of 25 and 70 years. A potential limitation of the study may be that their responses will possibly not be reflective of responses that might occur in the elderly who might have compromised health function.

The subject selection process was well established overall in the proposal through physical screening and informed-consent form. However, Ghio et al. presented a case of a 58-year-old woman who showed an increased risk for supraventricular arrhythmia and was later hospitalized overnight for observation. This event illustrates that physical screening and the informed-consent form cannot ensure there is zero risk of an adverse event occurring during a controlled exposure study.

Is the study method appropriate?

The study method is appropriate for testing the narrow hypotheses being tested:

- the order of the clean air and UFPM exposures was randomized,
- a double-blinded randomization was used to avoid bias,
- the health end points of interest were measured three times at the proper time, and
- measurement time was consistent to avoid variations within a day.

However, the maximum of the UF PM concentration was not controlled precisely, and a reasonable consideration of dropout of subjects was missing.

Was the choice of the study's exposure concentration and duration appropriate?

The rationale for the exposure concentration and duration are clearly stated in the proposal. The study subjects will be exposed to ultrafine particles concentrated from Chapel Hill air, typical concentrations range from 4,000 to 12,000 particles/cc, an instrument will concentrate the particles so that it is anticipat-

ed that the study subjects will be exposed to 11,000-330,000 UFP/cc on average and will establish a maximum of 600,000 particles/cc which is less than one would be exposed to driving a heavily traveled highway in Los Angeles.

This study was arranged as a 2-hour exposure duration, which seemed to follow previous study designs. The rationale for the 2-hour duration was not provided. Also, the subjects were sitting at rest with no activities during the 2-hour exposure time in this study. There could be differences in real exposure level with/without activity.

Was the choice of study end points appropriate for the experimental question?
Appropriate. The study outcomes are flow-mediated dilation (brachial artery ultrasound) and heart rate variability, peripheral venous blood samples, specific and nonspecific immune responses (cytokines and C-reactive protein), coagulation factors (von Willibrand factor, factor IX, fibrinogen and thrombin), vasoactive factors (endothelina, catecholamine), and soluble components of PM (transition metals).

Were the time points for measurement appropriate?
Appropriate. Three measurements, before exposure and 1 and 20 hours after the exposure. This would likely be sufficient to indicate the trend in rebound cardiovascular, inflammatory, and endothelial end points.

Was the statistical analysis appropriate?
Appropriate. The primary outcome will compare preexposure flow-mediated dilation to mean flow-mediated dilation 1 and 20 hours after the exposure. A second analysis will compare mean preexposure heart rate variability measures to the mean measures taken 1 and 20 hours after exposure. Both analyses will use an F-test to control for type I error, with flow-mediated dilation and HRV as separate dependent variables and exposure and time from exposure as independent variables. Although there are only 30 subjects to be recruited, they plan to use a repeated-measures ANOVA, and paired T-tests will be applied to the blood end points and Holter monitor analysis. With such a small sample size, nonparametric statistics might be more appropriate.

The sample size estimate for the research question is very low, 30; however, the investigators state that they expect to have 80% power to detect a difference of 3% in flow-mediated dilation assuming a SD of 4%. An N of 16 would give 80% power, $p < 0.05$; however, they are recruiting 30 subjects to allow for subject variability.

The analysis in the publication did not justify the assumption of a normal distribution for the ratio of two response measurements. Let Y1, Y2, and Y3 represent the response measured at time points 1 (baseline), 2, and 3, respectively. In the publication both ratios of Y2/Y1 and Y3/Y1 were assumed to have normal distributions. In general, the ratio of two responses, Y2/Y1, is *not* normal even if both Y1 and Y2 *are* normally distributed. As described in the proposal appendix, the study analysis should start with postulating the distribution of the responses or end points. There are a few methods available for handling a ratio of two correlated normal variables. As an alternative, a lognormal distribution can be assumed for the health end points, if applicable.

The publication also used a genetic factor from which the whole population (N=19) was compared to its subset, GSTM1 null population (N=9). For this small sample size of 9, nonparametric test methods are recommended.

As indicated in the publication, the study examined multiple responses from individual study subjects. Due to small sample size, the correlations among the multiple responses were not considered in the analysis.

What are the strengths and limitations of the study?
The strengths of this study can be regarded as threefold:

1. This study can generate a profile of a susceptible and/or sensitive subpopulation (people with metabolic syndrome), which can be used for future studies;
2. This study has information on the UF PM distribution in terms of number of particles; and
3. This study may separate UF PM effect from $PM_{2.5}$ effect.

Like most studies of this type, it involved a limited number of subjects. There was a lack of a rigorous statistical analysis of the uncertainties in estimates due to relatively small sample size (N=34 in total) and very small sample size for GSTM1 null population (N=9). However, if this subpopulation has a significant response to this exposure based on the outcome variables, additional studies will be warranted, and in addition, the EPA will have fulfilled its mission to discover subpopulations at risk for other diseases from ultrafine air pollutants.

What are the major findings of the study?
They reported in their publication as follows:

- Exposure to UF does not cause changes in brachial artery diameter or blood pressure.
- Exposure to UF causes changes in cardiac repolarization and heart rate variability.
- Exposure to UF causes changes in vascular markers of inflammation and fibrinolysis.
- The results suggest that UF may affect some biologic pathways by oxidative processes in which GSTM1 (an enzyme) may play a role.

The lack of BAD or BP responses may reflect the variability of the measure and lack of power, the specific content of these UFs (generalizability), subject susceptibility, or differences in the biologic effects of UF, compared to $PM_{2.5}$ in Chapel Hill.

What are the remaining uncertainties?
Like all studies of limited and prescribed exposures to volunteers, many questions remain. The study volunteers were between the ages of 25 and 70 years. Effects upon the very young and old were not investigated. Given that metabolic syndrome is more prevalent in individuals over the age of 50 years (44%), it might have been worthwhile to increase the enrollment of that age group. On the other hand, people younger than 70 often have metabolic syndrome without established CVD, whereas those older than 70 may be more likely to have overt clinical disease, which may be a contraindication for enrolling them.

Three results remain uncertain: (a) the results from GSTM1 null (N=9) due to small sample size, (b) UF effect with particle number or particle mass needs further investigation, and (c) UF effect and $PM_{2.5}$ effect are not comparable due to the use of different units of measurement (that is, particle number and particle mass).

Describe how the findings have or may contribute to the following aspects:
Quantification of the relationship between physiologic function and pollutant exposure.
The main measure for UF-induced change in responses was percent change in relative ratio to the individual baselines. This study quantified the changes in end points after exposure to UF, not the relationship between UF and end points.

Understanding biologic plausibility of effects of concern.
The results of this study, when completed, may determine if individuals with metabolic syndrome when exposed to UF PM have changes in the CV function, inflammation, and endothelial function and thrombosis that may over time increase their risk of development of cardiovascular disease.

Increased ability to interpret effects observed in toxicologic or epidemiologic studies.
The results from this study could possibly provide reinforcement to interpretations of effects observed in other studies and define a new at-risk subpopulation, individuals with metabolic syndrome.

Other: Contributions of publications out of the study.
There are two publications related to this study, Devlin et al. (2014) and Ghio et al. (2012). Devlin reported significant changes in HRV caused by UF PM with no significant changes in FMD and blood end points.

Ghio presented a case of a 58-year-old woman, who volunteered to participate in a controlled exposure to concentrated ambient particles and showed an increased risk for supraventricular arrhythmia. The authors claimed it the first case of cardiovascular disease after exposure to elevated concentrations of any air pollutant, which was not supported by Langrish et al. (2014). Langrish reported no changes from his controlled exposure experiment and indicated the atrial fibrillation (AF) as the most common cardiac arrhythmia in the general population and associated with increasing age, hypertension, and cardiac dysfunction. The authors suggest that in the case Ghio reported it is more likely that the investigators simply witnessed an asymptomatic episode of AF in a patient at increased arrhythmic risk due to coexistent hypertension, age, and frequent atrial ectopy, and the occurrence of AF in the exposure chamber is likely to have been coincidence and simply due to chance.

According to the Office of Inspector General report: "NHEERL management met and determined that no screening could have feasibly been done to have predicted this issue. The XCON consent form already warned study participants not to participate if they had cardiovascular disease including coronary artery disease, heart failure, or rhythm disturbances" (EPA 2014, page 27).

One other thing to note: antioxidant genes that are highly prevalent have been shown in a number of studies to increase risk of a variety of subclinical outcomes associated with pollution exposures. The 2014 published report considers GSTM1 null as a source of susceptibility to UF.

REFERENCES

Devlin, R.B., C.B. Smith, M.T. Schmitt, A.G. Rappold, A. Hinderliter, D. Graff, and M.S. Carraway. 2014. Controlled exposure of humans with metabolic syndrome to concentrated ultrafine ambient particulate matter causes cardiovascular effects. Toxicol. Sci. 140(1):61-72.

EPA (U.S. Environmental Protection Agency). 2008. Clean Air Research Multi-Year Plan 2008-2012. EPA 620/R-08/001.Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC. June 2008. https://permanent.access.gpo.gov/lps106873/Air-MYP-narrative-final.pdf [accessed March 3, 2017].

EPA. 2011. Policy Assessment for the Review of the Particulate Matter National Ambient Air Quality Standards. EPA/452/R-11-003. Office of Air Quality Planning and Standards, EPA, Research Triangle Park, NC. April 2011 [online]. Available: https://www3.epa.gov/ttn/naaqs/standards/pm/data/20110419pmpafinal.pdf [accessed March 3, 2017].

EPA. 2014. Improvements to EPA Policies and Guidance Could Enhance Protection of Human Study Subjects. Report No. 14-P-0154. Office of Inspector General. March 31, 2014 [online]. Available: https://www.epa.gov/sites/production/files/2015-09/documents/20140331-14-p-0154.pdf [accessed May 24, 2016].

Ghio, A.J., M. Bassett, T. Montilla, E.H. Chung, C.B. Smith, W.E. Cascio, and M.S. Carraway. 2012. Case report: Sypraventricular arrhythmia after exposure to concentrated ambient air pollution particles. Environ. Health Perspect. 120(2):275-277.

Hesterberg, T.W., C.M. Long, S.N. Sax, C.A. Lapin, R.O. McClellan, W.B. Bunn, and P.A. Valberg. 2011. Particulate matter in new technology diesel exhaust (NTDE) is quantitatively and qualitatively very different from that found in traditional diesel exhaust (TDE). J. Air Waste Manage. Assoc. 61(9):894-913.

Khalek, I.A., T.L. Bougher, P.M. Merritt, and b. Zielinska. 2011. Regulated and unregulated emissions from highway heavy-duty diesel engines complying with U.S. Environmental Protection Agency 2007 emissions standards. J. Air Waste Manag. Assoc. 61(4):427-442.

Khalek, I.A., M.G. Blanks, P.M. Merritt, and B. Zielinska. 2015. Regulated and unregulated emissions from modern 2010 emissions-compliant heavy-duty on-highway diesel engines. J. Air Waste Manag. Assoc. 65(8):987-1001.

Kuehl, R.O. 1994. Statistical Principles of Research Design and Analysis. Belmont, CA: Duxbury Press.

Langrish, J.P., S.J. Watts, A.J. Hunter, A.S.V. Shah, J.A. Bosson, J. Unosson, S. Barath, M. Lundbäck, F.R. Cassee, K. Donaldson, T. Sandström, A. Blomberg, D.E. Newby, and N.L. Mills. 2014. Controlled exposures to air pollutants and risk of cardiac arrhythmia. Environ. Health Perspect. 122(7):747-753.

Madden, M.C., T. Stevens, M. Case, M. Schmitt, D. Diaz-Sanchez, M. Bassett, T.S. Montilla, J. Berntsen, and R.B. Devlin. 2014. Diesel exhaust modulates ozone-induced lung function decrements in healthy human volunteers. Part. Fibre Toxicol. 11:37.

McClellan, R.O., T.W. Hesterberg and J.C. Wall. 2012. Evaluation of carcinogenic hazard of diesel engine exhaust needs to consider revolutionary changes in diesel technology. Regul. Toxicol. Pharmacol. 63(2): 225-258.

Romieu, I., M.M. Tellez-Rojo, M. Lazo, A. Manzano-Patino, M. Cortez-Lugo, P. Julien, M.C. Bélanger, M. Hernandez-Avila, and F. Holguin. 2005. Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. Am. J. Respir. Crit. Care Med. 172(12):1534-1540.

Stiegel, M.A., J.D. Pleil, J.R. Sobus, M.K. Morgan, and M.C. Madden. 2015. Analysis of inflammatory cytokines in human blood, breath condensate, and urine using a multiplex immunoassay platform. Biomarkers 20(1):35-46.

Stiegel, M.A., J.D. Pleil, J.R. Sobu, and M.C. Madden. 2016. Inflammatory cytokines and white blood cell counts response to environmental levels of diesel exhaust and ozone Inhalation exposures. PLoS One 11(4):e0152458.

Tong, H., A.G. Rappold, D. Diaz-Sanchez, S.E. Steck, J. Berntsen, W.E. Cascio, R.B. Devlin, and J.M. Samet. 2012. Omega-3 fatty acid supplementation appears to attenuate particulate air pollution–induced cardiac effects and lipid changes in healthy middle-aged adults. Environ. Health Perspect. 120(7):952-957.

Tong, H., A.G. Rappold, N. Caughney, A.L. Hinderliter, M. Bessett, T. Montilla, M.W. Case, J. Berntsen, P.A. Bromberg, W.E. Cascio, D. Diaz-Sanchez, R.B. Devlin, and J.M. Samet. 2015. Dietary supplementation with olive oil or fish oil and vascular effects of concentrated ambient particular matter exposure in human volunteers. Environ. Health Perspect. 123(11):1173-1179.

WHO (World Health Organization). 2015. Residential Heating with Wood and Coal: Health Impacts and Policy Options in Europe and North America [online]. Available: http://apps.who.int/iris/bitstream/10665/153671/1/ResidentialHeatWoodCoal.pdf [accessed March 3, 2017].